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(54) Title: RECOMBINANT AND HUMANIZED IL-1 β ANTIBODIES FOR TREATMENT OF IL-1 MEDIATED INFLAMMATORY DISORDERS IN MAN (57) Abstract This invention provides monoclonal and recombinant, chimeric and humanized antibodies directed to epitopes on the human protein, IL-1 β , compositions employing these antibodies, and methods for their preparation and use.		

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RECOMBINANT AND HUMANIZED IL-1 β ANTIBODIES FOR
TREATMENT OF IL-1 MEDIATED INFLAMMATORY DISORDERS IN MAN

Cross Reference to Related Applications

10 This application is a continuation-in-part of U.S. Serial No. 08/090,534 filed
July 9, 1993.

Field of the Invention

15 This invention relates generally to the field of monoclonal and
recombinant, humanized antibodies, and more specifically, to antibodies directed to
epitopes on the human protein, IL-1 β , compositions employing these antibodies, and
methods for their preparation and use.

Background of the Invention

20 Interleukin 1 (IL-1) is a protein mediator, or lymphokine, which is produced by
macrophages, monocytes, lymphocytes and other cells, generally when such cells are
themselves stimulated by various agents, e.g., endotoxins, cytokines, etc. IL-1, when
produced by these cells, stimulates the activities of many cells at the site of
inflammation, resulting in the further activation of lymphocytes, granulocytes and
25 fibroblasts. Thus, the production of IL-1 acts as a mediator of the acute phase
inflammatory response. Two proteins which share IL-1 activity are referred to as IL-1
 α and IL-1 β . For a current review of the activities and uses of the IL-1 cytokine, see,
e.g., C. A. Dinarello and R. C. Thompson, Immunol. Today, 12:404-410 (1991); see
also, March et al., Nature, 315:641-647 (1985).

30 IL-1 β has been cloned and found to have a precursor protein [P. Auron et al,
Proc. Natl. Acad. Sci. USA, 81:7907-7911 (1984) and C. March et al, Nature,
315:641-647 (1985)]. The mature IL-1 β protein begins at amino acid residue 117
(Ala) of the precursor protein.

35 A variety of antibodies have been disclosed which are directed to epitopes on
the IL-1 β molecule. For example, a number of monoclonal antibodies directed against
human IL-1 β are described. See, e.g., European Patent Application Publication No.
267,611, published May 18, 1988; European Patent Application Publication No.
364,778, published April 25, 1990; U. S. Patent No. 4,935,343, issued June 19, 1990;
International Patent Application Publication No. WO88/09508, published December 1,
40 1988; International Patent Application Publication No. WO90/06371, published June
14, 1990; International Patent Application Publication No. WO91/00742, published

- 5 January 24, 1991; and European Patent Application Publication No. 374,510, published June 27, 1990.

Chimeric monoclonal antibodies to lymphokines, including IL-1 β and monocyte-derived lymphocyte activating factor, have also been described. See, e.g., International Patent Application Publication No. WO91/14438, published October 3,
10 1991.

When IL-1, and particularly IL-1 β , is produced in excess quantities, it can contribute to the pathology of such disorders as septicemia, septic or endotoxic shock, chronic allergies, asthma, chronic rheumatoid arthritis, ischemia, stroke and other inflammatory disorders. IL-1 β antibodies have been described as useful in the
15 treatment of such disorders. See, Wolfe Editorial, New Engl. J. Med., 324(7):486-488 (1991); Wakabayashi, FASEB J., 5:338-343 (1991); Bone, JAMA, 266(12):1686-1691 (1991); Calandra, "Anti-lipopolysaccharide and anti-tumor necrosis factor/cachectin antibodies for the treatment of gram-negative bacteremia and septic shock" in Sturk, A. *et al.*, eds. Bacterial Endotoxins, Cytokine Mediators and New
20 Therapies for Sepsis; and Proceedings of the Third International Conference on Endotoxins, Progress in Clinical and Biological Research, 367:141-159 (1991).

However, the use of murine or other animal antibodies for treatment of inflammatory and other conditions in heterologous species, particularly in humans, is limited by the immune response of these species to the foreign, e.g., murine, antibody.
25 For example, immune responses in humans against murine antibodies have been shown to both immunoglobulin constant and variable regions.

Several techniques have been described which suggest alteration of murine (and other species) antibodies to reduce the occurrence of an immune response in a desired species, e.g., human, to the parent antibody [PCT Patent Application No.
30 PCT/GB85/00392; British Patent Application Publication No. GB2188638A; Amit *et al.*, Science, 233:747-753 (1986); Queen *et al.*, Proc. Nat'l. Acad. Sci. USA, 86:10029-10033 (1989); PCT Patent Application No. PCT/WO9007861; and Riechmann *et al.*, Nature, 332:323-327 (1988)]. While the prior art suggests possible experimental techniques, none show how to provide the combination of properties
35 required for preservation of effective prevention of IL-1 mediated inflammatory diseases *in vivo*.

However, there remains a need in the art to specifically identify neutralizing antibodies that are suitable pharmaceuticals capable of blocking the IL-1 β ligand and also protection against IL-1 mediated inflammatory diseases.

5 Summary of the Invention

 In one aspect, the invention provides a murine monoclonal antibody (mAb), SK48-E26, which is a neutralizing antibody specific for human recombinant interleukin-1 β , a functional fragment such as a Fab fragment or a F(ab')₂ fragment, an analog or modification thereof.

10 In another aspect, the present invention provides complementarity determining region (CDR) peptides from a human IL-1 β monoclonal antibody, fragments, analogs or modifications of the peptides. Particularly disclosed are the CDRs from the murine monoclonal antibody SK48-E26 and modifications to the heavy chain CDR region, for example, CDR3.

15 In another aspect, the present invention provides an isolated nucleic acid sequence encoding at least one CDR-encoding sequence of the present invention. The nucleic acid sequence may encode an entire antibody sequence, or fragments, analogs or modifications thereof. The nucleic acid sequence may be part of a recombinant plasmid and may be transformed or transfected into a host cell.

20 In yet a further aspect, the invention provides the nucleotide and amino acid sequences [SEQ ID NOS: 1 and 2] of the V_H and C_H1 regions (Fd) of the heavy chain of the cDNA clone of mAb SK48-E26 or fragments, such as the variable region and CDRs thereof, or analogs or modifications thereof.

25 In still a further aspect, the invention provides the nucleotide and amino acid sequences [SEQ ID NO: 3 and 4] of the light chain of the cDNA clone of mAb SK48-E26, fragments, such as the variable region and CDRs thereof, and analogs or modifications thereof.

30 In another aspect, the present invention provides a fusion protein comprising the amino acid sequence of a variable light chain and/or heavy chain of mAb SK48-E26, an anti IL-1 β CDR, or a functional fragment or analog or modifications thereof, operatively linked to a selected fusion partner. These fusion proteins are characterized by the antigen binding specificity of the antibody from which the CDR or heavy or light chain variable region, or functional fragment or analog or modification is derived. Particularly disclosed are the CDRs from the murine monoclonal antibody SK48-E26 and modifications to the heavy chain CDR region, for example, CDR3.

35 As yet an additional aspect, the invention provides nucleic acid sequences which encode all or a portion of the fusion proteins of this invention.

 In still a further aspect, the present invention provides an engineered neutralizing antibody with specificity for IL-1 β , in which at least parts of the CDRs in

5 the light and/or heavy chain variable domains of an acceptor antibody have been replaced by analogous parts of CDRs from the antibody SK48-E26 described herein, which have specificity for human IL-1 β .

In another aspect, the invention provides an IL-1 β antibody, a Fab fragment or a F(ab')₂ fragment thereof produced by screening an antibody library comprising
10 hybridoma products and libraries from any species immunoglobulin repertoires, derived from mAb SK48-E26.

As another aspect, the invention also provides methods for producing the fusion proteins, engineered antibodies and Fab and F(ab')₂ fragments described herein, including methods for producing them in selected host cells. Such methods may
15 employ other aspects of the invention, i.e., a plasmid vector containing nucleotide sequences encoding the fusion protein under the control of regulatory sequences capable of directing the replication and expression thereof in selected host cells, and host cells transfected with same.

Yet another aspect of this invention relates to a pharmaceutical composition
20 comprising a therapeutic amount of at least one such fusion protein, engineered antibody, or Fab or F(ab')₂ fragments described herein and a pharmaceutically acceptable carrier or diluent.

As another aspect a method of prophylactically or therapeutically treating an IL-1 β -mediated related inflammatory condition in a human or animal in need thereof is
25 provided. The method comprises administering an effective amount of at least one such fusion protein, monoclonal antibody, engineered antibody, Fab or F(ab')₂ fragment or modification thereof, to such human or animal.

In another aspect, the present invention relates to a method of diagnosing IL-1 β -mediated disorders comprising the step of contacting a biological sample from a
30 human or animal suspected of having an IL-1 β -mediated disorder with the protein(s) of this invention (e.g., fusion protein, monoclonal antibody, engineered antibody, Fab or F(ab')₂ fragment or modification thereof). Also embodied is a diagnostic kit for the diagnosis IL-1 β -mediated disorders.

Other aspects and advantages of the present invention are described further in
35 the following detailed description of preferred embodiments thereof.

Brief Description of the Drawings

Fig. 1 is a graph demonstrating the neutralizing effect of purified SK48-E26 vs. IL-1 α (open circle) and IL-1 β (open triangle) by bioassay. This figure shows the dose

5 dependent inhibition of IL-1 β induced IL-1 production (but not IL-1 α) by the purified antibody.

Fig. 2 is a graph demonstrating the results of an ELISA for human IL-1 β using a rabbit anti-human IL-1 β polyclonal antibody as the detection antibody and the neutralizing mAb SK48-E26 as the capture antibody. The level of detection was
10 between 0.1 to 0.2 ng/mL. A linear relationship was observed between the concentration of IL-1 and the corresponding optical density reading at 405 nm in the ELISA for IL-1 β .

Fig. 3 is a schematic drawing of plasmid pIL1hzhcpCD employed to express a synthetic IL-1 β heavy chain in mammalian cells. The plasmid contains a beta
15 lactamase gene (BETA LAC), an SV-40 origin of replication (SV40), a cytomegalovirus promoter sequence (CMV), a signal sequence, the synthetic variable heavy chain of SEQ ID NOS: 5 and 6, a human heavy chain constant region, a poly A signal from bovine growth hormone (BGH), a betaglobin promoter (beta glopro), a dihydrofolate reductase gene (DHFR), and another BGH sequence poly A signal in a
20 pUC19 background.

Fig. 4 is a schematic drawing of plasmid pIL1hzlcpCDN employed to express the synthetic IL-1 β light chain variable region of SEQ ID NOS: 7 and 8 in mammalian cells. The plasmid differs from that of Fig. 3 by containing a synthetic humanized light chain variable region rather than the synthetic heavy chain, a human light chain
25 constant region and a neomycin gene (Neo) in place of DHFR.

Detailed Description of the Invention

The present invention provides a variety of antibodies or fragments thereof, and fusion proteins, particularly humanized antibodies, which are characterized by IL-1 β
30 binding specificity and preferably the same or enhanced neutralizing activity as the murine monoclonal antibody (mAb) SK48-E26. These products are useful in therapeutic and pharmaceutical compositions for treating IL-1 β -mediated inflammatory disorders. These products are also useful in the diagnosis of an IL-1 β mediated pathology by measurement, e.g., by ELISA, of circulating, endogenous IL-1 levels *in*
35 *vivo* in humans.

I. Definitions.

As used herein, the term "first fusion partner" refers to a nucleic acid sequence encoding an amino acid sequence, which can be all or part of a heavy chain, a light

5 chain, functional fragment thereof including the variable region from one or both chains and CDRs therefore, or analog thereof, having the antigen binding specificity of a selected antibody, preferably the IL-1 β antibody, SK48-E26.

As used herein the term "second fusion partner" refers to another nucleotide sequence encoding a protein or peptide to which the first fusion partner is fused in
10 frame or by means of an optional conventional linker sequence. Such second fusion partners may be heterologous to the first fusion partner. A second fusion partner may include a nucleic acid sequence encoding a second antibody region of interest, e.g., all or part of an appropriate human constant region or framework region.

The term "fusion molecule" refers to the product of a first fusion partner
15 operatively linked to a second fusion partner. "Operative linkage" of the fusion partners is defined as an association which permits expression of the antigen specificity of the anti-IL-1 β sequence (the first fusion partner) from the donor antibody as well as the desired characteristics of the second fusion partner. For example, a nucleic acid sequence encoding an amino acid linker may be optionally used, or linkage may be via
20 fusion in frame to the second fusion partner.

The term "fusion protein" refers to the result of the expression of a fusion molecule in a selected host cell. Such fusion proteins may be engineered antibodies, e.g., chimeric antibodies, humanized antibodies, or any of the antibody regions identified herein fused to other immunoglobulin or non-immunoglobulin proteins and
25 the like.

As used herein, the term "donor antibody" refers to an antibody (polyclonal, monoclonal, or recombinant) which contributes the nucleic acid sequences of its naturally-occurring or modified light and/or heavy chains, variable regions thereof, CDRs thereof or other functional fragments or analogs thereof to a first fusion partner,
30 so as to provide the fusion molecule and resulting expressed fusion protein with the antigenic specificity or neutralizing activity characteristic of the donor antibody. A donor antibody suitable for use in this invention is murine IL-1 β antibody SK48-E26.

As defined herein, the murine IL-1 β antibody SK48-E26 is defined as an antibody having the light chain DNA and amino acid sequences of SEQ ID NO: 3 and
35 4, and the heavy chain (V_H and C_H1) DNA and amino acid sequences of SEQ ID NO: 1 and 2 on a suitable murine IgG framework. Such suitable framework regions may be identified through the literature, commercial sources, or from databases, e.g., the KABAT database, Los Alamos database, and Swiss Protein database, or isolated from an appropriate hybridoma as is described in Example 1 below.

5 As used herein the term "acceptor antibody" refers to an antibody (polyclonal, monoclonal, or recombinant) heterologous to the donor antibody, but homologous to the patient (human or animal) to be treated, which contributes all or any portion of the nucleic acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to a second fusion partner. Preferably a human antibody is an acceptor antibody.

"CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains which provide the majority of contact residues for the binding of the antibody to the antigen or epitope. CDRs of interest in this invention are derived from donor antibody variable heavy and light chain sequences, and include functional fragments and analogs of the naturally occurring CDRs, which fragments and analogs also share or retain the same antigen binding specificity and/or neutralizing ability as the donor antibody from which they were derived. By 'sharing the antigen binding specificity or neutralizing ability' is meant, for example, that although mAb SK48-E26 may be characterized by a certain level of antigen affinity, and a CDR encoded by a nucleic acid sequence of SK48-E26 in an appropriate structural environment may have a lower or higher affinity, it is expected that CDRs of SK48-E26 in such environments will nevertheless recognize the same epitope(s) as SK48-E26.

A "functional fragment" is a partial CDR sequence or partial heavy or light chain variable sequence or Fab or F(ab')₂ fragment which retains the same antigen binding specificity, and preferably the same neutralizing ability, as the antibody from which the fragment was derived.

An "analog" is an amino acid sequence altered by the deletion and/or replacement of at least one amino acid, or chemical modification of an amino acid, which alteration permits the amino acid sequence to retain the biological characteristics, e.g., antigen specificity, antigen avidity, etc., of the unmodified sequence.

An "allelic variation or modification" is an alteration in the nucleic acid sequence encoding the amino acid or peptide sequences of the invention. Such variations or modifications may be due to degeneracies in the genetic code or may be deliberately engineered (e.g., site-directed mutagenesis) to provide desired characteristics. When the variations or modifications do not result in the alteration of encoded amino acid sequence(s), the biological characteristics of the resultant antibody (or fusion protein or peptide), e.g., antigen specificity, antigen avidity, etc., are the

5 same as the wild-type or unmodified sequence. When the modifications (or variations) result in the alteration of encoded amino acid sequence(s), the biological characteristics of the resultant antibody, e.g., antigen specificity, antigen avidity, etc., are the same, and preferably are enhanced, as compared to the wild-type or unmodified sequence.

As used herein, an "engineered antibody" describes a type of fusion protein,
10 i.e., a synthetic antibody (e.g., a chimeric or humanized antibody) in which a portion of the light and/or heavy chain variable domains of a selected acceptor antibody are replaced by analogous parts of CDRs from one or more donor antibodies which have specificity for the selected epitope. These engineered antibodies may also be characterized by alteration of the nucleic acid sequences encoding the acceptor
15 antibody light and/or heavy variable domain framework regions in order to retain donor antibody binding specificity. These antibodies can comprise immunoglobulin (Ig) constant regions and variable framework regions from the acceptor antibody, and one or more CDRs from the IL-1 β donor antibody described herein.

A "chimeric antibody" refers to a type of engineered antibody which contains
20 naturally-occurring variable region light chain and heavy chains (both CDR and framework regions) derived from a non-human donor antibody in association with light and heavy chain constant regions derived from a human (or other heterologous animal) acceptor antibody.

A "humanized antibody" refers to an engineered antibody having its CDRs
25 and/or other portions of its light and/or heavy variable domain framework regions derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one or more human immunoglobulins. Such antibodies can also include engineered antibodies characterized by a humanized heavy chain associated with a donor or acceptor
30 unmodified light chain or a chimeric light chain, or vice versa.

The term "effector agents" refers to non-immunoglobulin carrier molecules to which the fusion proteins, and/or natural or synthetic light or heavy chain of the donor antibody or other fragments of the donor antibody may be associated by conventional means. Such non-protein carriers can include conventional carriers used in the
35 diagnostic field, e.g., polystyrene or other plastic beads, or other non-protein substances useful in the medical field and safe for administration to humans and animals. Other effector agents may include a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin. Such effector agents are useful to increase the half-life of the anti-IL-1 β derived amino acid sequences or to add to its properties.

5

II. IL-1 β Antibodies

For use in constructing the antibodies, fragments and fusion proteins of this invention, a non-human species may be employed to generate a desirable immunoglobulin upon presentment with the active human IL-1 β molecule or a peptide epitope therefrom. Conventional hybridoma techniques are employed to provide a hybridoma cell line secreting a non-human monoclonal antibody (mAb) to the IL-1 β protein. As one example, the production of murine mAb SK48-E26 is described in detail in Example 1 below. SK48-E26 is a desirable donor antibody for use in developing a chimeric or humanized antibody of this invention. Other mAbs generated against a desired IL-1 β epitope and produced by conventional techniques, include without limitation, genes encoding murine mAbs, human mAbs, and combinatorial antibodies.

The characteristics of the neutralizing murine mAb SK48-E26 obtained as described in Example 1 include an antigen binding specificity for active, human recombinant IL-1 β (hrIL-1 β) or naturally occurring IL-1 β . SK48-E26 binds to IL-1 β at a conformational epitope of the hrIL-1 β molecule which includes amino acids #95-101 [SEQ ID NO: 11] of IL-1 β , which is adjacent to the Type I receptor binding site of IL-1 β [See, e.g., C. A. Dinarello and R. C. Thompson, Immunol. Today, 12:404-410 (1991)]. It has been demonstrated that monoclonal antibodies which are directed against this epitope are neutralizing *in vitro*. The binding is illustrated by binding and functional activity (neutralization) in an *in vitro* assay (see Examples 2 and 3 below).

The isotype of the mAb SK48-E26 of Example 1 is IgG₁, and it has an affinity for IL-1 β of between about 0.5 and 3 nM, depending on the assay employed. The antibody recognizes a conformational epitope on IL-1 β and does not recognize denatured IL-1 β . Several potential epitopes were suggested by Geysen et al, Proc. Natl. Acad. Sci. (USA), 81:3998-4002 (1984), via scanning peptide analysis with the primary epitope from amino acids 95-101 [SEQ ID NO: 11]. See also, Geysen et al, Mol. Immunol., 23:709-715 (1986).

Given the sequences provided, i.e., the light chain of SK48-E26 [SEQ ID NO: 3 and 4] and the heavy chain variable and the first portion of the constant region [SEQ ID NOS: 1 and 2], one of skill in the art could obtain the remaining portions of the heavy chain using, for example, polymerase chain reaction, and thus obtain a complete mAb molecule. Alternatively, a SK48-E26 molecule could be constructed using techniques analogous to those described below for the synthetic and recombinant

- 5 mAbs of the invention and employing other murine IgG subtype heavy chains, e.g., IgG₂ or IgG₄.

Various peptide epitopes of the IL-1 β protein may elicit additional mAbs and CDRs useful in prophylactic agents against IL-1 β -mediated inflammatory disorders. The IL-1 β epitope spanning amino acids #95-101 [SEQ ID NO: 11] to which SK48-E26 is responsive, and analogs thereof, is anticipated to be useful in the screening and development of additional IL-1 β antibodies, which are anticipated to be similarly useful in this invention.

For example, other IL-1 β antibodies may be developed by screening hybridomas or combinatorial libraries, or antibody phage displays [W. D. Huse *et al.*, *Science*, 246:1275-1281 (1988)] using the murine mAb described herein and its IL-1 β epitope. A collection of antibodies, including hybridoma products or antibodies derived from any species immunoglobulin repertoire may be screened in a conventional competition assay, such as described in Example 2 below, with one or more epitopes described herein. Thus, the invention may provide an antibody, other than SK48-E26, which is capable of binding to and neutralizing the IL-1 β molecule, more specifically to the conformational epitope containing amino acid #95-101 [SEQ ID NO: 11] and analogs and modifications thereof.

This invention is not limited to the use of the SK48-E26 mAb or its hypervariable sequences. It is anticipated that any appropriate IL-1 β neutralizing antibodies and corresponding anti-IL-1 β CDRs described in the art may be substituted therefor. Wherever in the following description the donor antibody is identified as SK48-E26, this designation is made for illustration and simplicity of description only.

III. Antibody Fragments

30 The present invention also includes the use of Fab fragments or F(ab')₂ fragments derived from mAbs directed against an epitope of IL-1 β as agents protective *in vivo* against IL-1 β mediated inflammatory diseases. A Fab fragment contains the entire light chain and F_d, which consists of the amino terminal portion of the heavy chain (V_H and C_H1) and part of the hinge region. A F(ab')₂ fragment is the fragment formed by two Fab fragments bound by disulfide bonds. MAb SK48-E26, or other similar IL-1 β binding and neutralizing antibodies, provide a source of Fab fragments and F(ab')₂ fragments which can be obtained by conventional means, e.g., cleavage of the mAb with the appropriate proteolytic enzymes, papain and/or pepsin, or by recombinant methods. For example, a Fab fragment of mAb SK48-E26 is provided by

- 5 SEQ ID NO: 4 (light chain) and SEQ ID NO: 2 ($V_H + C_H1$). These Fab and $F(ab')_2$ fragments are useful themselves as therapeutic, prophylactic or diagnostic agents, and as donors of sequences including the variable regions and CDR sequences useful in the formation of recombinant or humanized antibodies as described herein.

10 *IV. Anti-IL-1 β Amino Acid and Nucleotide Sequences of Interest*

- The mAb SK48-E26 or other antibodies described above may contribute sequences, such as variable heavy and/or light chain peptide sequences, framework sequences, CDR sequences, functional fragments, modifications and analogs thereof, and the nucleic acid sequences encoding them, useful in designing and obtaining
15 various fusion proteins (including engineered antibodies) which are characterized by the antigen binding specificity of the donor antibody.

- As one example, the present invention thus provides isolated naturally-occurring or synthetic variable light chain and variable heavy chain sequences derived from the IL-1 β antibody mAb SK48-E26, as shown in SEQ ID NOS: 1-8. The V_H
20 and C_H1 portions of the naturally-occurring heavy chain clone of SK48-E26 is characterized by the amino acid and encoding nucleic acid sequences illustrated in SEQ ID NOS: 2 and 1, respectively. The nucleotide region of the heavy chain variable region spans nucleotide #226-582 of SEQ ID NO: 1. The amino acid region of the heavy chain variable region spans amino acids #20 through 138 of SEQ ID NO: 2.
25 The remainder of the sequence includes some 5' untranslated sequence as well as the native signal sequence and only part of the conserved regions. The variable heavy region is specifically described in Example 4, part B, below.

- The naturally occurring light chain clone of SK48-E26 is characterized by the amino acid sequence and encoding nucleic acid sequence of SEQ ID NOS: 4 and 3,
30 respectively. Its variable region spans nucleotides #93-413 of SEQ ID NO: 3. The amino acid region of the light chain variable region spans amino acids #21 through 127 of SEQ ID NO: 4. The remainder of the sequence includes the light chain conserved region and some 5' untranslated sequence as well as the native signal sequence. See, Example 4, part B, below.

- 35 Synthetic, humanized heavy chain variable region nucleotide and amino acid sequences are illustrated in SEQ ID NOS: 5 and 6. An exemplary humanized light chain variable sequence is illustrated in SEQ ID NOS: 7 and 8. The heavy chain and the light chain variable regions of SEQ ID NOS: 1-4 have three CDR sequences described in detail in Example 4.

5 The nucleic acid sequences of this invention, or fragments thereof, encoding the variable light chain and heavy chain peptide sequences or CDR peptides, or functional fragments thereof are used in unmodified form or are synthesized to introduce desirable modifications. The isolated naturally-occurring or synthetic nucleic acid sequences, which are derived from mAb SK48-E26 or from other desired IL1 β antibodies may optionally contain restriction sites to facilitate insertion or ligation into a suitable nucleic acid sequence encoding a desired antibody framework region, ligation with mutagenized CDRs or fusion with a nucleic acid sequence encoding a selected second fusion partner.

10 Taking into account the degeneracy of the genetic code, various coding sequences may be constructed which encode the variable heavy and light chain amino acid sequences, and CDR sequences of the invention and functional fragments and analogs thereof which share the antigen specificity of the donor antibody. The isolated or synthetic nucleic acid sequences of this invention, or fragments thereof, encoding the variable chain peptide sequences or CDRs or functional fragments thereof can be used to produce fusion proteins, chimeric or humanized antibodies, or other engineered antibodies of this invention when operatively combined with a second fusion partner.

15 These sequences are also useful for mutagenic introduction of specific changes within the nucleic acid sequences encoding the CDRs or framework regions, and for incorporation of the resulting modified, or fusion, nucleic acid sequence into a vector for expression. For example, silent nucleotide substitutions were made in the nucleotide sequence encoding CDR2 of the human heavy and light chain variable regions described below to create restriction enzyme sites used to facilitate insertion of mutagenic framework regions. These regions were used in the construction of a humanized antibody of this invention. Other modifications were made in the nucleotide sequence encoding CDR3 of the human heavy chain to create antibodies with increased affinity for IL1 β . Positions 121 and 122 are the most tolerant of substitutions. Positions 119-124 of SEQ ID NO: 2 were mutagenized. For antibodies with increased affinity for IL-1 β , it is preferable that positions 119 and 123 are glycine, position 120 is valine and position 124 is tyrosine. Preferred modifications to position 121 include arginine (R), histidine (H) and threonine (T). Preferred modifications to position 122 include arginine (R) or lysine (K).

5 V. *Engineered Antibodies and Other Fusion Molecules*

10 Fusion proteins can include engineered antibodies, chimeric antibodies, and humanized antibodies. A desired fusion protein contains a first fusion partner sequence encoding a peptide having the antigen specificity and neutralizing activity of an IL-1 β antibody such as SK48-E26 and analogs thereof, operatively linked to a second fusion
15 partner. Engineered antibodies directed against functional fragments or analogs of IL-1 β may be designed to elicit enhanced binding with the same antibody.

15 The second fusion partners are defined above, and may include a sequence encoding a peptide or protein such as a second antibody region of interest. Second fusion partners may also include sequences encoding another protein or peptide to
20 which the light or heavy chain is fused in frame or by means of a linker sequence.

20 The first fusion partners may also be associated with effector agents as defined above, including non-protein carrier molecules, to which the first fusion partner may be operatively linked by conventional means.

25 Fusion or linkage between the first fusion partners, e.g., the IL-1 β antibody sequences, and the selected second fusion partner may be by any suitable means, e.g., by conventional covalent or ionic bonds, protein fusions, or hetero-bifunctional cross-linkers, e.g., carbodiimide, glutaraldehyde, and the like. Where the first fusion partner is associated with an effector agent, non-proteinaceous, conventional chemical linking agents may be used to fuse or join the anti-IL-1 β sequences to the effector agent. Such
30 techniques are known in the art and readily described in conventional chemistry and biochemistry texts.

30 Additionally, conventional linker sequences which simply provide for a desired amount of space between the fusion partners or between the first fusion partner and the effector agent may also be constructed into the fusion molecule. The design of
35 such linkers is well known to those of skill in the art.

35 Expression of such fusion molecules results in fusion proteins of this invention. As one example a desired fusion protein contains an amino acid sequence of a naturally occurring heavy chain sequence of SEQ ID NO: 2, a functional fragment, modification or an analog thereof, a naturally occurring light chain sequence of SEQ ID NO: 4, a
40 functional fragment, modification or analog thereof. Preferably, the functional fragments of these heavy and light chains are their respective variable regions.

40 Another exemplary fusion protein contains a synthetic variable heavy and/or light chain peptide or protein sequence having the antigen specificity and similar neutralizing activity of mAb SK48-E26, e.g., those of SEQ ID NOS: 6 and 8. Still

- 5 another desirable fusion protein of this invention is characterized by the amino acid sequence containing at least one, and preferably all of the CDRs of the variable region of the heavy and/or light chains of the murine antibody molecule SK48-E26, or a functional fragment, modification or analog thereof (see Example 4, below).

10 In still a further embodiment, the engineered antibody (or the other monoclonal antibodies) of the invention may have attached to it an effector agent. For example, the procedure of recombinant DNA technology may be used to produce an engineered antibody of the invention in which the F_C fragment or CH3 domain of a complete antibody molecule has been replaced by an enzyme or toxin molecule. An example of a fusion protein of this invention provides the anti-IL-1 β sequence of the invention
15 associated with a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to the SK48-E26 encoding nucleic acids by a covalent bridging structure.

When the second fusion partner is a second peptide, protein or fragment thereof heterologous to the CDR-containing sequence having the antigen specificity of SK48-E26, the resulting fusion protein may exhibit anti-IL-1 β antigen specificity,
20 neutralizing activity, and the characteristic of the second fusion partner upon expression. That fusion partner characteristic may be, e.g., a functional characteristic such as secretion from a recombinant host, or a therapeutic characteristic if the fusion partner is itself a therapeutic protein, or additional antigenic characteristics, if the
25 fusion protein has its own antigen specificity.

Another fusion protein of this invention may comprise a complete antibody molecule, having full length heavy and light chains, or any fragment thereof, such as the Fab or F(ab')₂ fragment, a heavy chain dimer, or any recombinant fragment thereof such as an F_V or a single-chain antibody (SCA) or any other molecule with the same
30 specificity, and preferably neutralizing activity, as the selected donor mAb, e.g., the mAb SK48-E26. Alternatively, one or more of these fragments may be used in an unfused form.

If the fusion partner is derived from another antibody, e.g., any isotype or class of immunoglobulin framework or constant region, an engineered antibody results. In
35 an engineered antibody at least fragments of the variable heavy and/or light domains of an acceptor antibody have been replaced by analogous parts of the variable light and/or heavy chains from one or more donor antibodies. These engineered antibodies can comprise immunoglobulin (Ig) constant regions and variable framework regions from one source, e.g., the acceptor antibody, and one or more CDRs from the donor

5 antibody, e.g., the IL-1 β antibody described herein. In addition, alteration, e.g., deletions, substitutions, or additions, of the acceptor mAb light and/or heavy variable domain framework region, or the CDRs, at the nucleic acid or amino acid levels may be made in order to retain donor antibody antigen binding specificity.

Such engineered antibodies are designed to employ one or more of the variable
10 heavy or light chains of the IL-1 β mAb (optionally modified as described) or one or more of the below-identified heavy or light chain CDR amino acid and encoding nucleic acid sequences (see Example 4). The engineered antibodies desirably block binding to the receptor of the IL-1 β protein. Specifically, the engineered antibody is directed against a specific tertiary protein epitope of human IL-1 β , which includes
15 amino acid #95-101 [SEQ ID NO: 11], as described herein for SK48-E26.

Such engineered antibodies include a humanized antibody containing the framework regions of a selected human immunoglobulin or subtype, or a chimeric antibody containing the human heavy chain constant regions fused to the IL-1 β antibody functional fragments. A suitable human (or other animal) acceptor antibody
20 may be one selected from a conventional database, e.g., the KABAT database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable
25 framework region for the insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions (i.e., acceptor antibody chain) may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody.

30 Desirably the heterologous framework and constant regions are selected from human immunoglobulin classes and isotypes, such as IgG (subtypes 1 through 4), IgM, IgA, and IgE. However, the acceptor antibody need not comprise only human immunoglobulin protein sequences. For instance a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA
35 sequence encoding the amino acid sequence of a polypeptide effector or reporter molecule.

One example of a particularly desirable humanized antibody contains all or a portion of the variable domain amino acid sequences of SK48-E26 and some portions of the donor antibody framework regions, or CDRs therefrom inserted onto the

5 framework regions of a selected human antibody. This humanized antibody is directed against an IL-1 β epitope, preferably the epitope containing amino acids 95-101 [SEQ ID NO: 11] of huIL-1 β . Suitably, in these humanized antibodies one, two or preferably three CDRs from the IL1 β antibody heavy chain and/or light chain variable regions are inserted into the framework regions of a selected human antibody,
10 replacing the native CDRs of that latter antibody.

Preferably, in a humanized antibody, the variable domains in both human heavy and light chains have been engineered by one or more CDR replacements. It is possible to use all six CDRs, or various combinations of less than the six CDRs. For example, it is possible to replace the CDRs only in the human heavy chain, using as
15 light chain the unmodified light chain from the human acceptor antibody. Still alternatively, a compatible light chain may be selected from another human antibody by recourse to the conventional antibody databases. The remainder of the engineered antibody may be derived from any suitable acceptor human immunoglobulin.

The engineered humanized antibody thus preferably has the structure of a
20 natural human antibody or a fragment thereof, and possesses the combination of properties required for effective therapeutic use, e.g., treatment of IL-1 β mediated inflammatory diseases in man.

As another example, an engineered antibody may contain three CDRs of the variable light chain region of SK48-E26 or a functional fragment thereof in place of at
25 least a part of the light chain variable region of an acceptor mAb, and three CDRs of the variable heavy chain region of SK48-E26 or a functional fragment thereof in place of at least a part of the heavy chain variable region of an acceptor mAb, such as a human antibody. The resulting humanized antibody is characterized by the antigen binding specificity of mAb SK48-E26.

30 It will be understood by those skilled in the art that an engineered antibody may be further modified by changes in variable domain amino acids without necessarily affecting the specificity of the donor antibody. It is anticipated that heavy and light chain amino acids may be substituted by other amino acids either in the variable domain frameworks or CDRs or both.

35 A fusion protein which is a chimeric antibody differs from the humanized antibodies described above by providing the entire non-human donor antibody heavy chain and light chain variable regions, including framework regions, e.g., amino acids 20 to 138 of SEQ ID NO: 2 and amino acids 21 to 127 of SEQ ID NO: 4, in association with human immunoglobulin constant regions for both chains. It is

- 5 anticipated that chimeric antibodies which retain additional non-human sequence in comparison to humanized antibodies of this invention may also elicit immune response in a human.

Such engineered antibodies are effective in the prevention and treatment of IL-1 mediated inflammatory disorders in humans and other animals. Humanized,
10 monoclonal antibodies which neutralize the biological activity of endogenous IL-1 β are useful therapeutically or prophylactically for such IL-1 β mediated disorders. Such disorders include those identified above, such as septic shock, arthritis and the like.

VI. Production of Fusion Proteins and Engineered Antibodies

- 15 Preferably the fusion proteins and engineered antibodies of the invention will be produced by recombinant DNA technology using genetic engineering techniques. The same or similar techniques may also be employed to generate other embodiments of this invention, e.g., to construct the chimeric or humanized antibodies, the synthetic light and heavy chains, the CDRs, the Fabs, and the nucleic acid sequences encoding
20 them, as above mentioned.

- A specific embodiment of the compositions of this invention is set out in Example 1 below using the CDRs of murine SK48-E26 and one or more selected human antibody light and heavy chain framework regions. This exemplary humanized antibody is characterized by the humanized heavy chain variable and light chain
25 variable sequences reported in SEQ ID NOS: 5 through 8. Briefly described, a hybridoma producing the murine antibody SK48-E26 is conventionally cloned, and the cDNA of its heavy and light chain variable regions obtained by techniques known to one of skill in the art, e.g., the techniques described in Sambrook *et al.*, Molecular Cloning (A Laboratory Manual), 2nd edition, Cold Spring Harbor Laboratory (1989).
30 The variable regions of the SK48-E26 are obtained using polynucleotide primers and reverse transcriptase. The CDRs are identified using a known database and by comparison to other antibodies.

- Homologous framework regions of a heavy chain variable region from a human antibody were identified using computerized databases, e.g., KABAT, and a human
35 antibody having homology to SK48-E26 was selected as the acceptor antibody. The sequences of synthetic heavy chain variable regions containing the SK48-E26 CDRs within the human antibody frameworks are designed with optional nucleotide replacements in the framework regions to incorporate restriction sites [SEQ ID NOS: 5 and 6]. This designed sequence is then synthesized by overlapping oligonucleotides,

5 amplified by polymerase chain reaction (PCR), and corrected for errors.

A suitable light chain variable framework region [SEQ ID NOS: 7 and 8] was designed in a similar manner.

10 These synthetic variable light and/or heavy chain sequences and the CDRs of mAb SK48-E26, and their encoding nucleic acid sequences, are utilized in the construction of fusion proteins and engineered antibodies, preferably humanized antibodies, of this invention, by the following process. By conventional techniques, a DNA sequence is obtained which encodes the donor antibody variable heavy or light chain regions containing at least the CDRs and those minimal portions of the acceptor mAb light and/or heavy variable domain framework region required in order to retain donor mAb binding specificity, as well as the remaining immunoglobulin-derived parts of the antibody chain derived from a human immunoglobulin.

15 A conventional expression vector or recombinant plasmid is produced by placing these coding sequences for the fusion protein in operative association with conventional regulatory control sequences capable of controlling the replication and expression in, and/or secretion from, a host cell. Such regulatory sequences may be readily selected by one of skill in the art and are not intended as a limitation of the present invention. Regulatory sequences include promoter sequences, e.g., CMV promoter, and signal sequences (e.g., SEQ ID NOS: 9 and 10), which can be derived by one of skill in the art from antibodies. Similarly, a second expression vector is produced having a DNA sequence which encodes a complementary antibody light or heavy chain. Preferably this second expression vector is identical to the first except in so far as the coding sequences and selectable markers are concerned so to ensure as much as possible that each polypeptide chain is functionally expressed.

25 In another alternative, a single vector of the invention may be used, the vector including the sequence encoding both light chain and heavy chain-derived polypeptides. The DNA in the coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both.

30 A selected host cell is co-transfected by conventional techniques with both the first and second vectors or simply transfected by a single vector to create the transfected host cell of the invention comprising both the recombinant or synthetic light and heavy chains. The transfected cell is then cultured by conventional techniques to produce the engineered antibody of the invention. The humanized antibody which includes the association of both the recombinant heavy chain and/or light chain is screened from culture by appropriate assay, such as the ELISA assay

5 described in Example 2 below. Similar conventional techniques may be employed to construct other fusion proteins and molecules of this invention.

Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by one of skill in the art. For example, the conventional pUC series of cloning vectors, may be used.
10 One vector used is pUC19, which is commercially available from supply houses, such as Amersham (Buckinghamshire, United Kingdom) or Pharmacia (Uppsala, Sweden). Additionally, any vector which is capable of replicating readily, has an abundance of cloning sites and marker genes, and is easily manipulated may be used for cloning. Thus, the selection of the cloning vector is not a limiting factor in this invention.

15 Similarly, the vectors employed for expression of the engineered antibodies according to this invention may be selected by one of skill in the art from any conventional vector. The vectors also contain selected regulatory sequences which are in operative association with the DNA coding sequences of the immunoglobulin regions and capable of directing the replication and expression of heterologous DNA
20 sequences in selected host cells, such as CMV promoters. These vectors contain the above described DNA sequences which code for the engineered antibody or fusion molecule. Alternatively, the vectors may incorporate the selected immunoglobulin sequences modified by the insertion of desirable restriction sites for ready manipulation.

25 The expression vectors may also be characterized by marker genes suitable for amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR) or neomycin resistance gene (neo^R). Other preferable vector sequences include a poly A signal sequence, such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The
30 expression vectors useful herein may be synthesized by techniques well known to those skilled in this art.

The components of such vectors, e.g. replicons, selection genes, enhancers, promoters, signal sequences and the like, may be obtained from natural sources or synthesized by known procedures for use in directing the expression and/or secretion
35 of the product of the recombinant DNA in a selected host. Other appropriate expression vectors of which numerous types are known in the art for mammalian, bacterial, insect, yeast, and fungal expression may also be selected for this purpose.

Two exemplary expression vectors employed in the following examples for expression of the IL-1 β fusion proteins of this invention are the mammalian vectors

5 illustrated in Figs. 3 and 4. However, this invention is not limited to the use of these illustrative vectors.

The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the engineered antibodies or fusion molecules hereof. Host cells useful for the cloning and other manipulations of these cloning vectors are also conventional. However, most desirably, cells from
10 various strains of *E. coli* are used for replication of the cloning vectors and other steps in the construction of fusion proteins of this invention.

Suitable host cells or cell lines for the expression of the engineered antibody or fusion protein of the invention are preferably a eukaryotic cell, and most preferably a
15 mammalian cell, such as a CHO cell or a myeloid cell. Other primate cells may be used as host cells and, most desirably, human cells are used, thus enabling the molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be employed. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and
20 purification are known in the art. See, e.g., Sambrook *et al.*, cited above.

Bacterial cells may prove useful as host cells suitable for the expression of the recombinant mAbs of the present invention. However, due to the tendency of proteins expressed in bacterial cells to be in an unfolded or improperly folded form or in a non-glycosylated form, any recombinant mAb produced in a bacterial cell would have to be
25 screened for retention of antigen binding ability. If the molecule expressed by the bacterial cell was produced in a properly folded form, that bacterial cell would be a desirable host. For example, various strains of *E. coli* used for expression are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Streptomyces*, other bacilli and the like may also be employed in this method.

30 Where desired, strains of yeast cells known to those skilled in the art are also available as host cells, as well as insect cells (e.g., S₂, Sf9) and viral expression systems. See, e.g. Miller *et al.*, Genetic Engineering, 8:277-298, Plenum Press (1986) and references cited therein.

The general methods by which the vectors of the invention may be constructed,
35 transfection methods required to produce the host cells of the invention, and culture methods necessary to produce the fusion protein or engineered antibody of the invention from such host cell are all conventional techniques. Likewise, once produced, the fusion proteins or engineered antibodies of the invention may be purified from the cell culture contents according to standard procedures of the art, including

5 ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Such techniques are within the skill of the art and do not limit this invention.

Yet another method of expression of the humanized antibodies may utilize expression in a transgenic animal, such as described in U. S. Patent No. 4,873,316.
10 This relates to an expression system using the animal's casein promoter which when transgenically incorporated into a mammal permits the female to produce the desired recombinant protein in its milk.

Once expressed by the desired method, the engineered antibody is then examined for *in vitro* activity by use of an appropriate assay. Presently conventional
15 ELISA assay formats are employed to assess qualitative and quantitative binding of the engineered antibody to the IL-1 β epitope.

Following the procedures described for humanized antibodies prepared from this antibody, one of skill in the art may also construct humanized antibodies from other IL-1 β antibodies, variable region sequences and CDR peptides described herein.
20 Engineered antibodies can be produced with variable region frameworks potentially recognized as "self" by recipients of the engineered antibody. Modifications to the variable region frameworks can be implemented to effect large increases in antigen binding without appreciable increased immunogenicity for the recipient. Such engineered antibodies can effectively treat a human for IL-1 mediated inflammatory
25 diseases. Such antibodies may also be useful in the diagnosis of such diseases.

VII. *Therapeutic/Prophylactic Uses of the Invention*

This invention also relates to a method of treating humans experiencing an IL-1 β mediated inflammatory disorder which comprises administering an effective dose of
30 antibodies including one or more of the engineered antibodies or fusion proteins described herein, or fragments thereof.

The therapeutic response induced by the use of the engineered antibodies of this invention is produced by the binding of the immunoglobulin to the IL-1 β molecule and the subsequent sequestering and/or clearance of this bound complex. Thus, the
35 engineered antibodies of the present invention, when in preparations and formulations appropriate for therapeutic use, are highly desirable for those persons experiencing an IL-1 β mediated inflammatory response, such as an allergy, rheumatoid arthritis, septic or endotoxic shock, septicemia, asthma, graft versus host disease, Crohn's disease, and other inflammatory bowel diseases.

5 The fusion proteins, antibodies, engineered antibodies, modifications or fragments thereof of this invention may also be used in conjunction with other antibodies, particularly human mAbs reactive with other markers (epitopes) responsible for the disease against which the engineered antibody of the invention is directed. Similarly mAbs reactive with epitopes responsible for the disease in a selected animal
10 against which the antibody of the invention is directed may also be employed in veterinary compositions. Any antibody that is capable of operating without interfering with the IL1 β antibody of this invention, e.g., an antibody to other IL-1 β epitopes, an antibody to IL-1 α epitopes, etc. is useful in these compositions.

 The therapeutic agents of this invention are believed to be desirable for
15 treatment of inflammatory conditions for from about 2 days to about 3 weeks, or as needed. This represents a considerable advance over the currently used infusion protocol with prior art treatments of IL-1 β mediated disorders, such as septic shock. This duration of treatment relates to the relative duration of the recombinant antibodies of the present invention in the human circulation.

20 The mode of administration of the therapeutic agent of the invention may be any suitable route which delivers the agent to the host. The fusion proteins, antibodies, engineered antibodies, and fragments thereof, and pharmaceutical compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly or intravenously. However, the agent is preferably
25 administered by i.v. injection, depending on the condition treated.

 Therapeutic agents of the invention may be prepared as pharmaceutical compositions containing an effective amount of the engineered or chimeric antibody of the invention as an active ingredient in a nontoxic and sterile pharmaceutically acceptable carrier. In the prophylactic agent of the invention, an aqueous suspension
30 or solution containing the engineered antibody, preferably buffered at physiological pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a solution of the engineered antibody of the invention or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., water, buffered water,
35 0.4% saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antibody of the invention

5 in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection
10 could be prepared to contain 1 mL sterile buffered water, and between about 50 to about 100 mg of an engineered antibody of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 150 mg of an engineered antibody of the invention. Actual methods for preparing parenterally administrable
15 compositions are well known or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania.

It is preferred that the therapeutic agent of the invention, when in a pharmaceutical preparation, be present in unit dose forms. The appropriate
20 therapeutically effective dose can be determined readily by those of skill in the art. To effectively treat an inflammatory disorder in a human or other animal, one dose of approximately 1 mg/kg to approximately 20 mg/kg of a protein or an antibody of this invention should be administered parenterally, preferably i.v. (intravenously). Such dose may, if necessary, be repeated at appropriate time intervals during the
25 inflammatory response.

The invention also encompasses the administration of the IL-1 β fusion proteins of this invention concurrently or sequentially with other antibodies or fusion proteins characterized by anti-IL-1 α activity, anti-tumor necrosis factor activity or other pharmaceutical activities compatible with the IL-1 β receptor binding ability of the
30 fusion proteins of this invention. Such other antibodies are available commercially or can be designed in a manner similar to that described herein.

The fusion proteins and engineered antibodies of this invention may also be used in diagnostic regimens, such as for the determination of IL-1 β mediated disorders or teaching progress of treatment of such disorders. As diagnostic reagents, these
35 fusion proteins may be conventionally labeled for use in ELISA's and other conventional assay formats for the measurement of IL-1 levels in serum, plasma or other appropriate tissue. The nature of the assay in which the fusion proteins are used are conventional and do not limit this disclosure.

The antibodies, engineered antibodies or fragments thereof described herein

5 can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

Single or multiple administrations of the pharmaceutical compositions can be carried out with dose levels and pattern being selected by the treating physician. In
10 any event, the pharmaceutical composition of the invention should provide a quantity of the engineered antibodies of the invention sufficient to effectively treat the patient.

The following examples illustrate various aspects of this invention including the construction of exemplary engineered antibodies and expression thereof in suitable vectors and host cells, and are not to be construed as limiting the scope of this
15 invention. All amino acids are identified by conventional three letter or single letter codes. All necessary restriction enzymes, plasmids, and other reagents and materials were obtained from commercial sources unless otherwise indicated. All general cloning ligation and other recombinant DNA methodology were as performed in T. Maniatis *et al.*, cited above, or the second edition thereof (1989), eds. Sambrook *et al.*, by the same publisher ("Sambrook *et al.*").
20

Example 1 - Production of mAb SK48-E26

A. Immunization procedure

Balb/c mice (females) were immunized with *E. coli*-expressed human
25 recombinant IL-1 β protein [C. A. Meyers et al, J. Biol. Chem., 262:11176-11181 (1987)]. The immunization schedule is described in Table 1 below. Various amounts of protein were injected into mice via i.p., s.c. and i.v. routes. The antigen was presented in different types of adjuvants to maximize the immune stimulation. The immunization protocol varied for each injection, since simple immunization procedures
30 were not successful in generating neutralizing IL-1 β monoclonal antibodies. Over 108 mAbs against hrIL-1 β were generated with binding properties. However, they did not have neutralizing activities.

For this experiment, the mice were injected with a combination of adjuvants, e.g., Incomplete Freund's Adjuvant, Complete Freund's Adjuvant, and
35 monophosphoryl lipid A (MPL) + TDM emulsion (0.5 mg each in 2% oil-Tween-80-H₂O) (Catalog #060-130; Ribi Immunochem, Hamilton, MT) and via different routes. Three days prior to fusion (as routinely used in the generation of murine monoclonal antibodies) the mice were injected i.v. The immunization resulted in high neutralizing antibody titers, as detected in the serum of immunized mice.

5

Table 1
Immunization Schedule

Day	Immunogen	Adjuvants	Route of Injection	Quantity of IL-1 β
1	hrIL-1 β	ICFA/CFA	s.c.	20 μ g
46	hrIL-1 β	Ribi	i.p.	10 μ g
73	hrIL-1 β	None	i.v.	10 μ g

10

B. Fusion Procedure and Screening System

Three mice were sacrificed on Day 76 and single cell suspensions were prepared from their spleens. A total of 1.7×10^8 spleen cells were fused with 1.7×10^7 SP-2/0-Ag14 cells (ATCC CRL1581; murine myeloma fusion partner; ratio 10:1). The procedure for the fusion was routine, using polyethylene glycol and DMSO as fusogenic reagents [R. H. Kennett, "Fusion Protocol" in *Monoclonal Antibodies. Hybridomas: A New Dimension in Biological Analyses*. T. J. McKearn and K. B. Bechtol, eds., Plenum Press, NY, pp. 365-367 (1983)]. A total of eight 96-well plates were plated (2×10^5 cells per well). The cells were subjected to HAT selection pressure 24 hours after fusion. The fusion resulted in a total of 86 hybridoma clones.

20

Example 2 - ELISA

Antibody-secretion was detected in supernatants from the hybridomas of Example 1 in an ELISA measuring binding to hrIL-1 β 10-25 days after fusion. A standard ELISA, as essentially described by R. H. Kennett "Enzyme linked antibody assay with cells attached to polyvinyl chloride plates" in *Monoclonal Antibodies. Hybridomas: A New Dimension in Biological Analyses*. T. J. McKearn and K. B. Bechtol, eds., Plenum Press, NY, pp. 376-377 (1983) with the following modifications. The ELISA plates were coated with hrIL-1 β , 100 μ L/well were suspended in 0.1 M sodium bicarbonate buffer (pH 9.6) at a concentration of 5 μ g/mL and the o-phenylenediamine substrate was incubated for 10 minutes at room temperature.

30

O.D. was measured by using a Multiskan MC ELISA reader (Flow Laboratories, McLean, VA) at 492 nm. The results are illustrated in Fig. 2. The positive hybridoma clones were tested in the neutralization assay (EL-4 assay) of

5 Example 3.

Example 3 - Biological Activity

The following neutralizing assay is described in detail in P. L. Simon et al, J. Immunol. Meth., 84:85-94 (1985). Briefly described, biological activity was measured
10 by the ability of IL-1 β to stimulate the production of IL-2 in cultured murine EL-4 lymphoma cells (ATCC TIB39) in the presence of the calcium ionophore A23187. IL-2 production was detected by the incorporation of [³H] thymidine into the IL-2 dependent cytotoxic T-cell line CTLL-20 [S. Gillis and K. Smith, Nature, 268:154 (1977)].

15 One hybridoma clone designated as SK48-E26 was identified as a clone which produced mAbs with positive binding to IL-1 β in the ELISA assay of Example 2 and this neutralizing (EL-4 bioassay) activity (Fig. 1). The original hybridoma was subcloned twice (by limited dilution method) in order to assure monoclonality and also shows the activity described above.

20 The characteristics of the neutralizing monoclonal antibody SK48-E26 include a specificity for human recombinant IL-1 β , as determined in these assays. Its isotype is IgG₁, and it has an affinity of between about 1 and 3 nM, depending on the way that affinity is measured. SK48-E26 presumably bound to the conformational epitope which includes amino acids #95-101 of the hrIL-1 β molecule (MEKRFVF SEQ ID NO: 11),
25 which was identified as the major epitope by Geysen mapping.

This hybridoma produced on average 60-70 μ g/mL of specific IgG₁ antibodies from confluent cultures (T75 flasks, HY medium, 13% FCS). In serum-free medium the cells produced approximately 25 μ g/mL.

30 Example 4 - Humanized Antibodies

A humanized version of the IL-1 β specific mouse antibody, SK48-E26, was prepared by performing the following manipulations. Briefly, cDNA clones were made of the SK48-E26 heavy and light chains (Part A). DNA sequencing thereof determined that these clones were antibody genes (Part B). A mouse/human chimeric
35 antibody was then prepared and assayed for binding ability (Part C). To prepare a humanized antibody, human framework regions were selected and CDRs from the SK48-E26 heavy and light chains were inserted appropriately within the selected heavy and light chain framework. There may be subsequent manipulation of framework residues (Part D). The resulting humanized antibody was expressed in mammalian

5 cells, e.g., COS cells (Part E).

A. cDNA Cloning

cDNA clones were made of the SK48-E26 heavy and light chains from mRNA extracted out of the SK48-E26 hybridoma cell line [SmithKline Beecham] using a Boehringer Mannheim kit. Primers specific for either the mouse heavy chain hinge region or kappa constant region were used for first strand synthesis.

The kappa chain primer is [SEQ ID NO: 12]:

5' CTAACACTCATTCCTGTTGAAGCTCTTGACAATGGG 3'.

The heavy chain primer is [SEQ ID NO: 13]:

5' GTACATATGCAAGGCTTACAACCACAATC 3'.

15 The double stranded cDNA was cloned directly into plasmids pGEM7 [Promega] that were then transformed into *E.coli* DH5- α [Bethesda Research Labs].

B. DNA Sequencing

One each heavy and light chain cDNA clone from Part A above was sequenced. The results are shown in Fig. 1 (heavy chain) [SEQ ID NO: 1] and Fig. 2 (light chain) [SEQ ID NO: 3]. Each clone contained amino acids known to be conserved among mouse heavy chain constant regions or light chain constants regions, and murine signal sequences. The CDR amino acid sequences are listed below.

The nucleotide and amino acid numbers referred to below may be found within SEQ ID NOS: 1 and 2, at the appropriate nucleotide or amino acid position. A cloning adaptor spans nucleotides #1-14. The 5' untranslated region spans nucleotides #15-168. The naturally occurring variable heavy chain signal sequence spans nucleotides #169-225 and amino acids #1 to 19. Framework region 1 spans nucleotides #226-315, and amino acids #20-49. The first CDR spans nucleotides #316-330, amino acids #50-54 (SYDMS). The second framework region spans nucleotides #331-372, amino acids #55-68. The second CDR spans nucleotides #373-423, amino acids #69-85 (YISSGGGGTYYPDTVKG). The third framework region spans nucleotide #424-519, amino acids #86-117. The third CDR spans nucleotides #520-549, amino acids #118-127 (GGVRRGYFDV). The fourth framework region spans nucleotide #550-582, amino acids #128-138. A portion of the heavy chain constant region spans nucleotides #583-909, including amino acids #139-247. A cloning adaptor spans nucleotides #910-923.

The nucleotide and amino acid numbers referred to below may be found within SEQ ID NOS: 3 and 4 at the appropriate nucleotide or amino acid position. A

5 cloning adaptor spans nucleotides #1-14. The 5' untranslated region spans nucleotides
#15-32. The signal sequence spans nucleotides #33-92, and amino acids #1 to 20
thereof. Framework region 1 spans nucleotides #93-161, amino acids #21-43. The
first CDR spans nucleotides #162-194, amino acids #44-54 (RASGNIHNYLT). The
second framework region spans nucleotides #195-239, amino acids #55-69. The
10 second CDR spans nucleotides #240-260, amino acids #70-76 (NAKTLAD). The
third framework region spans nucleotide #261-356, amino acids #77-108. The third
CDR spans nucleotides #357-383, amino acids #109-117 (QHFWSIPYT). The fourth
framework region spans nucleotide #384-413, amino acids #118-127. The light chain
constant region spans nucleotides #414-737 and includes amino acids #128-234. A
15 cloning adaptor spans nucleotides #738-751.

C. Mouse/Human Chimeric Antibody

DNA coding for the native signal sequence and variable region of the
heavy and light chain of SK48-E26 (see SEQ ID NOS: 1 and 3) was ligated to a
20 human IgG1 constant region or human kappa constant regions contained within
plasmids pCD and pCDN (Figs. 3 and 4) designed for expression in mammalian cells.
These plasmids were then co-transfected by conventional means and expressed in COS
cells.

Media supernatants were collected three and five days later and assayed
25 by the ELISA described as follows: ELISA plates were coated with 0.1 µg/well of a
goat antibody specific for the Fc region of human antibodies. The media supernatants
were added for one hour. A horseradish peroxidase conjugated goat antibody specific
for an entire human IgG antibody was added. This was followed by addition of ABTS
peroxidase substrate (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD) for
30 one hour. O.D. was measured using a THERMO MAX microplate reader (Molecular
Devices Corporation, Menlo Park, California) at 405 nm.

Expression of the chimeric antibody was detected. In a second ELISA
the COS-cell supernatants containing the chimeric antibody bound specifically to
recombinant human IL-1β protein. This result confirmed that genes coding for an
35 antibody specific for IL-1β had been cloned.

D. Selection of Human Frameworks

For each of the SK48-E26 heavy and light chains, six to twelve human
antibody chains with high overall amino acid sequence homology to the SK48-E26
heavy and light chains were selected from the KABAT® database. The best match of

5 these was then detected using a method that weighs the impact that changes in the
framework residues may have on the structure of a CDR. Given the murine SK48-E26
CDRs and the sequence of the chosen human antibody, a synthetic heavy chain variable
region was designed to replace the naturally occurring CDRs of the human framework
sequence with the CDRs of SK48-E26. In designing the sequence, conservative
10 nucleotide replacements were placed in the framework regions to introduce selected
restriction sites suitable for enzymatic cleavage.

The human heavy chain was described in the reference I. W. Schmidt,
et al., "Die Primärstruktur des kristallisierbaren monoklonalen Immunglobulins IgG1
KOL", Hoppe-Seyler's Z. Physiol. Chem., Number 364, p. 713 (1983). The light
15 chain was published in W. Palm and H. Hilschmann, "Die Primärstruktur einer
kristallinen monoklonalen Immunglobulin-L-Kette vom k-Typ, Subgruppe I (Bence-
Jones-Portein Rei.) ein Beitrag zur Aufklärung der dreidimensionalen Struktur der
Immunglobuline.", Hoppe-Seyler's Z. Physiol. Chem., Number 354, p. 1651 (1973).
The framework regions of these human antibodies were determined to be
20 approximately 74% and 76% identical in amino acid sequence to the SK48-E26
variable heavy chain and light chain framework regions, respectively.

The humanized variable regions were synthesized using a combination
of overlapping oligonucleotides and PCR amplification. Any errors in the designed
sequences which were inserted by PCR were corrected. The humanized heavy chain
25 and light chain variable region DNA and protein sequences are shown in SEQ ID
NOS: 5 through 8.

E. Expression of Humanized mAb

pUC18 subclones were made to add a signal sequence [SEQ ID NOS: 9
and 10]. To produce an exemplary humanized antibody, DNA fragments containing
30 this signal linked to either the humanized heavy or light variable regions were inserted
into pUC19-based mammalian cell expression plasmids containing CMV promoters
and human heavy chain or human light chain constant regions by conventional methods
[Maniatis *et al.*, cited above] to yield the plasmids p111hzhcpCD (heavy chain) and
p111hzcpcDN (light chain).

35 These plasmids are co-transfected into COS cells and supernatants assayed by
the ELISA described immediately above for the presence of human antibody after three
and five days.

5 Example 5 - Modification of SK48-E26

Mutagenesis (random and site-directed) of SK48-E26 was performed as described below. Briefly, DNA encoding the antibody light chain and antibody Fd chain (comprising the heavy-chain variable region and heavy-chain constant region domain 1 (CH₁)) of SK48-E26 was inserted into a phage display vector. The CDR3
10 region of the heavy chain of SK8-E26 was modified and antibodies with increased affinity for IL1 β were further selected for characterization.

A. Sub-cloning a SK48-E26 Fab

The vector pMK or pComb 3 [Barbas et al., Proc. Natl. Acad. Sci.
15 USA, 88:7978-7982 (1991)] can be used for cloning, periplasmic expression, and M13 phage display of Fab antibody fragments.

Light chain

The light chain DNA from SK48-E26 (see Example 4A) was PCR amplified using the
20 following primers [SEQ ID NO: 14 and 15, respectively]:

5' CTCCGCGTCGACGAGCTCCAGATGACTCAGTCTCC 3'

The underlined region is a Sst I site.

5' CTCCGCGTCGACTCTAGACCTAACACTCATTTTC 3'

The underlined region is a Xba I site.

25 The PCR fragment was digested with Sst I and Xba I, followed by isolation of the digested product from an acrylamide gel. The fragment was then ligated into Sst I, Xba I digested pMK vector DNA, and the ligation reaction was used to transform *E. coli* XL-1 blue by electroporation. Insertion of the SK48 light chain fragment was confirmed by restriction digestion. The resultant plasmid codes for an SK48 light
30 chain in which the amino-terminal end is fused to the pel B leader sequence. As a result of this sub-cloning the first 2 amino-acids of the light chain (positions 21 and 22, SEQ ID NO: 4) were changed from A, I to E, L respectively.

Heavy chain

35 The heavy chain DNA from SK48-E26 was PCR amplified using the following primers [SEQ ID NO: 16 and 17, respectively]:

5' CTCCGCGTCGACCTCGAGTCTGGGGGAGGC 3'

The underlined region is a Xho I site.

5' CTCCGCGTCGACACTAGTACAATCCCTGGGCAC 3'

- 5 The underlined region is an Spe I site.

The PCR fragment was digested with Xho I and Spe I, followed by isolation of the digested product from an acrylamide gel. The fragment was then ligated into Xho I, Spe I digested pMK vector DNA (already containing the SK48-E26 light chain), and the ligation reaction was used to transform *E. coli* XL-1 blue by electroporation.

- 10 Insertion of the SK48-E26 heavy chain (Fd) fragment was confirmed by restriction digestion. The resultant plasmid (pMK-SK48) codes for an SK48-E26 heavy chain in which the amino-terminal end is fused to the pel B leader sequence, and the carboxyl-terminal end is fused to the M13 gene III anchor domain. As a result of this sub-cloning the first, third and fifth amino-acid positions of the heavy chain (positions 20, 22, and 24, SEQ ID NO: 2) were changed from E, H, V to Q, K, L respectively.
- 15 These N-terminal amino acid changes are not expected to significantly affect the binding affinity of the antibody.

B. Selection of Mutants with Increased Affinity

- 20 An Xma I site was introduced near CDR3 via site-directed mutagenesis with the following primer:

5' TCACTGTGCCCGGGGTGGTGTACGACGAGG 3' [SEQ ID NO: 18]

The amino acid sequence was not affected by this change. A library of CDR3H mutants was created using the following oligos as PCR primers.

- 25 5' GGTTGAGGCAGGTCAGACGATTGGC 3' [SEQ ID NO: 19] (anneals within the gene III region of pMK) and

5' TACTACTGTGCCCGGGGTGGTNNSNNSNNSGGGNNSTTCGATGTCTGG 3'
N = G, A, T, C; S = G or C only [SEQ ID NO: 20].

- The mutagenesis oligo was synthesized using a codon based strategy [Glaser et al., J. Immunol., 149:3903-3913 (1992)] in which there is a 50% chance that the targeted codon will not be wild-type. The targeted codons were chosen on the basis of previous mutagenesis experiments (Table 2) demonstrating they were tolerant of substitutions.
- 30

5

Table 2

Mutagenesis of SK48-E26 heavy chain CDR3

Wild -Type amino acid	% Mutagenized IL-1 binding clones	Mutations that reduce IL-1 binding
100G	18	A, C, M, R
101V	9	A, P, E, R
102R	47	A
103R	44	A, V
104G	11	E, R, V
105Y	32	A, G, P, I, D, T

- 10 Following amplification, the PCR fragment was digested with Xma I, and Spe I, ligated into pMK-SK48 vector DNA digested with the same enzymes and used to transform *E. coli* XL-1 blue. This was followed by expression of the Fab in the presence of VCSM13 helper phage which results in the library of Fabs being displayed on phage particles. This library is random with respect to amino acid identity at
- 15 positions 101, 102, 103, and 105 of the heavy chain (positions 120, 121, 122, and 124 of SEQ. ID NO: 1 or 2). The library was subjected to a Bio-panning selection process [Parmley and Smith, *Gene*, 73:305-318 (1988)] in 96 well microtiter plates coated with a range of 200 ng to 25 ng of IL-1 β per well. Several variations of the selection
- 20 process were performed including elution with acid, elution with soluble IL-1 β (2.4 mM), selection in the presence of a 10 fold molar excess of wild-type soluble SK48 Fab, and selection on IL-1 β conjugated agarose beads. Each variation is designed to select those phage with the highest affinity for IL-1 β . After 3 or 4 iterative rounds of selection were performed, in which the selected phage were re-amplified prior to the next selection, several clones were sequenced and several mutants were identified (see
- 25 Table 3). An additional mutant (R102T, R103K) was rationally designed and constructed by site-directed mutagenesis with the following oligo [SEQ ID NO: 21]: 5' GTGGTGTAACCAAGGGGTACTTCG 3'

5

Table 3

SK48-E26 CDR3H mutants selected from library

<u>101</u>	<u>102</u>	<u>103</u>	<u>105</u>
V	R	R	Y
V	T	R	Y
V	L	R	Y
V	R	K	Y
V	R	R	F
V	T	H	Y
V	Q	R	Y
I	R	R	Y
V	H	R	Y
V	H	K	Y
V	R	F	Y
V	R	K	W

bold-face indicates the wild-type sequence

10

C. Characterization of Mutant Fabs

Soluble expression is facilitated by the removal of the gene III fusion from the heavy chain [Barbas et al., Proc. Natl. Acad. Sci. USA, **88**:7978-7982 (1991)]. Several mutants were characterized for their ability to bind IL-1 β in solution via an ELISA assay [Friguet et al., J. Immunol. Methods, **77**:305-319 (1985)].

15

Purified Fab was mixed with various concentrations of IL-1 β and allowed to reach equilibrium. Subsequently the amount of free Fab remaining was measured via an IL-1 β mediated capture ELISA, and this value was used to calculate the amount of Fab and IL-1 β in complex at equilibrium. These values were plotted by the method of Klotz [The Proteins, Vol. 1, pp 727, H. Neurath and K. Bailey eds. (Academic Press, New York, 1953)] and the K_D of the various Fabs was determined from the slope of the plots. The K_D are presented in Table 4.

20

5

Table 4
Affinities of purified SK48-E26 mutants

<u>101</u>	<u>102</u>	<u>103</u>	<u>105</u>	<u>K_D (nM)</u>	<u>K_D wt/K_D mut</u>
V	R	R	Y	0.47 ± 0.085	1
V	T	R	Y	0.2 ± 0.029	2.35
V	T	K	Y	0.048 ± 0.012	9.79
V	R	K	Y	0.32	1.47
V	H	K	Y	0.29	1.62

bold-face indicates wild-type

10

The above examples describe the preparation of an exemplary engineered antibody and modification thereof. Similar procedures may be followed for the development of other engineered antibodies, using other IL1 β antibodies developed by conventional means.

15

Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. For example, human framework regions or modifications thereof, other than the exemplary antibodies described above, may be used in the construction of humanized antibodies. Such modifications and alterations to the compositions and processes of

20

the present invention are believed to be encompassed in the scope of the claims appended hereto.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Young, Peter
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Jonak, Zdenka L.
Theisen, Timothy
Hurle, Mark
Jackson, Jeffrey R.
- (ii) TITLE OF INVENTION: Recombinant and Humanized Il-1 beta
Antibodies for Treatment of Il-1 Mediated Inflammatory
Disorders in Man
- (iii) NUMBER OF SEQUENCES: 21
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 - (E) COUNTRY: USA
 - (F) ZIP: 19406-2799
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/090,534
 - (B) FILING DATE: 09-JUL-1993
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 923 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 169..909

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAATTCGCGG CCGCATGGTA TAGCAGGAAG ACATGCAAAT AAGTCTTCTC TGTGCCCATG	60
AAAAACACCT CGGCCCTGAC CCTGCAGCTC TGACAGAGGA GGCCAGTCCT GGAATTGATT	120
CCCAGTTCCT CACGTTCACT GATGAGCACT GAACACAGAC ACCTCACC ATG AAC TTT	177
Met Asn Phe	
1	
GGG CTC AGA TTG ATT TTC CTT GTC CTT ACT TTA AAA GGT GTG AAG TGT	225
Gly Leu Arg Leu Ile Phe Leu Val Leu Thr Leu Lys Gly Val Lys Cys	
5 10 15	
GAA GTG CAC CTG GTG GAG TCT GGG GGA GGC TTA GTG AAG CCT GGA GGG	273
Glu Val His Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly	
20 25 30 35	
TCC CTG AAA CTC TCC TGT GCA GCC TCT GGA TTC GCT TTC AGT AGC TAT	321
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ala Phe Ser Ser Tyr	
40 45 50	
GAC ATG TCT TGG GTT CGC CAG ACT CCG GAG AAG AGG CTG GAC TGG GTC	369
Asp Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Asp Trp Val	
55 60 65	
GCA TAC ATT AGT AGT GGT GGT GGT GGC ACC TAC TAT CCA GAC ACT GTG	417
Ala Tyr Ile Ser Ser Gly Gly Gly Gly Thr Tyr Tyr Pro Asp Thr Val	
70 75 80	
AAG GGC CGA TTC ACC ATC TCC AGG GAC AAT GCC AAG AAC ACC CTG TAC	465
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr	
85 90 95	
CTG CAA ATG AGC AGT CTG AAG TCT GAG GAC ACA GCC ATG TAT CAC TGT	513
Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr His Cys	
100 105 110 115	
GCA AGA GGG GGG GTA CGA CGA GGG TAC TTC GAT GTC TGG GGC GCA GGG	561
Ala Arg Gly Gly Val Arg Arg Gly Tyr Phe Asp Val Trp Gly Ala Gly	
120 125 130	
ACC ACG GTC ACC GTC TCC TCA GCC AAA ACG ACA CCC CCA TCT GTC TAT	609
Thr Thr Val Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr	
135 140 145	
CCA CTG GCC CCT GGA TCT GCT GCC CAA ACT AAC TCC ATG GTG ACC CTG	657
Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr Leu	
150 155 160	
GGA TGC CTG GTC AAG GGC TAT TTC CCT GAG CCA GTG ACA GTG ACC TGG	705
Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr Trp	
165 170 175	
AAC TCT GGA TCC CTG TCC AGC GGT GTG CAC ACC TTC CCA GCT GTC CTG	753
Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu	

180		185		190		195	
CAG TCT GAC CTC TAC ACT CTG AGC AGC TCA GTG ACT GTC CCC TCC AGC							801
Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser Ser							
		200		205		210	
ACC TGG CCC AGC GAG ACC GTC ACC TGC AAC GTT GCC CAC CCG GCC AGC							849
Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro Ala Ser							
		215		220		225	
AGC ACC AAG GTG GAC AAG AAA ATT GTG CCC AGG GAT TGT GGT TGT AAG							897
Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys Lys							
		230		235		240	
CCC TTG CAT ATG GCGGCCAGGA ATTC							923
Pro Leu His Met							
		245					

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 247 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Asn	Phe	Gly	Leu	Arg	Leu	Ile	Phe	Leu	Val	Leu	Thr	Leu	Lys	Gly
1				5					10					15	
Val	Lys	Cys	Glu	Val	His	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Lys
			20					25					30		
Pro	Gly	Gly	Ser	Leu	Lys	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Ala	Phe
			35				40					45			
Ser	Ser	Tyr	Asp	Met	Ser	Trp	Val	Arg	Gln	Thr	Pro	Glu	Lys	Arg	Leu
			50			55					60				
Asp	Trp	Val	Ala	Tyr	Ile	Ser	Ser	Gly	Gly	Gly	Gly	Thr	Tyr	Tyr	Pro
			65		70			75							80
Asp	Thr	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn
				85				90						95	
Thr	Leu	Tyr	Leu	Gln	Met	Ser	Ser	Leu	Lys	Ser	Glu	Asp	Thr	Ala	Met
			100					105					110		
Tyr	His	Cys	Ala	Arg	Gly	Gly	Val	Arg	Arg	Gly	Tyr	Phe	Asp	Val	Trp
			115				120					125			
Gly	Ala	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Ala	Lys	Thr	Thr	Pro	Pro
			130			135					140				
Ser	Val	Tyr	Pro	Leu	Ala	Pro	Gly	Ser	Ala	Ala	Gln	Thr	Asn	Ser	Met
			145		150				155						160
Val	Thr	Leu	Gly	Cys	Leu	Val	Lys	Gly	Tyr	Phe	Pro	Glu	Pro	Val	Thr

	165		170		175
Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro					
	180		185		190
Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val					
	195		200		205
Pro Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His					
	210		215		220
Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys					
	225		230		235
Gly Cys Lys Pro Leu His Met					
	245				

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 751 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 33..734

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAATTCGCGG CCGCCACTGA TCACACACAG TC ATG AGT GTG CTC ACT CAG GTC	53
Met Ser Val Leu Thr Gln Val	
1 5	
CTG GCG TTG CTG CTG CTG TGG CTT ACA GGT GCC AGA TGT GAC ATC CAG	101
Leu Ala Leu Leu Leu Leu Trp Leu Thr Gly Ala Arg Cys Asp Ile Gln	
10 15 20	
ATG ACT CAG TCT CCA GCC TCC CTA TCT GCA TCT GTG GGA GAA ACT GTC	149
Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Val Gly Glu Thr Val	
25 30 35	
ACC ATC ACA TGT CGA GCA AGT GGG AAT ATT CAC AAT TAT TTA ACA TGG	197
Thr Ile Thr Cys Arg Ala Ser Gly Asn Ile His Asn Tyr Leu Thr Trp	
40 45 50 55	
TAT CAG CAG AAA CAG GGA AAA TCT CCT CAG CTC CTG GTC TAT AAT GCA	245
Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Val Tyr Asn Ala	
60 65 70	
AAA ACC TTA GCA GAT GGT GTG CCG TCA AGG TTC AGT GGC AGT GGA TCA	293
Lys Thr Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser	
75 80 85	
GGA ACA CAA TAT TCT CTC AAG ATC AAC AGC CTG CAG CCT GAA GAT TTT	341
Gly Thr Gln Tyr Ser Leu Lys Ile Asn Ser Leu Gln Pro Glu Asp Phe	
90 95 100	

GGG AGT TAT TAC TGT CAA CAT TTT TGG AGT ATT CCG TAC ACG TTC GGA	389
Gly Ser Tyr Tyr Cys Gln His Phe Trp Ser Ile Pro Tyr Thr Phe Gly	
105 110 115	
GGG GGG ACC AAG CTG GAA ATA AAT CGG GCT GAT GCT GCA CCA ACT GTA	437
Gly Gly Thr Lys Leu Glu Ile Asn Arg Ala Asp Ala Ala Pro Thr Val	
120 125 130 135	
TCC ATC TTC CCA CCA TCC AGT GAG CAG TTA ACA TCT GGA GGT GCC TCA	485
Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly Gly Ala Ser	
140 145 150	
GTC GTG TGC TTC TTG AAC AAC TTC TAC CCC AAA GAC ATC AAT GTC AAG	533
Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile Asn Val Lys	
155 160 165	
TGG AAG ATT GAT GGC AGT GAA CGA CAA AAT GGC GTC CTG AAC AGT TGG	581
Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val Leu Asn Ser Trp	
170 175 180	
ACT GAT CAG GAC AGG AAA GAC AGC ACC TAC AGC ATG AGC AGC ACC CTC	629
Thr Asp Gln Asp Arg Lys Asp Ser Thr Tyr Ser Met Ser Ser Thr Leu	
185 190 195	
ACG TTG ACC AAG GAC GAG TAT GAA CGA CAT AAC AGC TAT ACC TGT GAG	677
Thr Leu Thr Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr Thr Cys Glu	
200 205 210 215	
GCC ACT CAC AAG ACA TCA ACT TCA CCC ATT GTC AAG AGC TTC AAC AGG	725
Ala Thr His Lys Thr Ser Thr Ser Pro Ile Val Lys Ser Phe Asn Arg	
220 225 230	
AAT GAG TGT TAGGCGGCCG CGAATTC	751
Asn Glu Cys	

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 234 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ser Val Leu Thr Gln Val Leu Ala Leu Leu Leu Trp Leu Thr	
1 5 10 15	
Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser	
20 25 30	
Ala Ser Val Gly Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Gly Asn	
35 40 45	
Ile His Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro	
50 55 60	
Gln Leu Leu Val Tyr Asn Ala Lys Thr Leu Ala Asp Gly Val Pro Ser	
39	

65					70						75				80
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Gln	Tyr	Ser	Leu	Lys	Ile	Asn
				85					90					95	
Ser	Leu	Gln	Pro	Glu	Asp	Phe	Gly	Ser	Tyr	Tyr	Cys	Gln	His	Phe	Trp
			100					105					110		
Ser	Ile	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Asn	Arg
		115					120					125			
Ala	Asp	Ala	Ala	Pro	Thr	Val	Ser	Ile	Phe	Pro	Pro	Ser	Ser	Glu	Gln
	130					135					140				
Leu	Thr	Ser	Gly	Gly	Ala	Ser	Val	Val	Cys	Phe	Leu	Asn	Asn	Phe	Tyr
145					150					155					160
Pro	Lys	Asp	Ile	Asn	Val	Lys	Trp	Lys	Ile	Asp	Gly	Ser	Glu	Arg	Gln
				165					170					175	
Asn	Gly	Val	Leu	Asn	Ser	Trp	Thr	Asp	Gln	Asp	Arg	Lys	Asp	Ser	Thr
			180					185					190		
Tyr	Ser	Met	Ser	Ser	Thr	Leu	Thr	Leu	Thr	Lys	Asp	Glu	Tyr	Glu	Arg
		195				200						205			
His	Asn	Ser	Tyr	Thr	Cys	Glu	Ala	Thr	His	Lys	Thr	Ser	Thr	Ser	Pro
	210					215					220				
Ile	Val	Lys	Ser	Phe	Asn	Arg	Asn	Glu	Cys						
225					230										

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 357 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..357

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAA	GTG	CAA	CTA	GTG	GAG	TCT	GGG	GGA	GGC	GTA	GTG	CAG	CCT	GGA	AGG	48
Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Val	Val	Gln	Pro	Gly	Arg	
1				5					10					15		
AGT	CTA	CGA	CTC	TCC	TGT	TCG	AGC	TCT	GGA	TTC	ATT	TTC	AGT	AGC	TAT	96
Ser	Leu	Arg	Leu	Ser	Cys	Ser	Ser	Ser	Gly	Phe	Ile	Phe	Ser	Ser	Tyr	
			20					25					30			
GAC	ATG	TCT	TGG	GTT	CGC	CAG	GCC	CCG	GGG	AAG	GGG	CTG	GAG	TGG	GTC	144
Asp	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	
			35				40					45				
GCA	TAC	ATT	AGT	TCC	GGA	GGT	GGT	GGC	ACC	TAC	TAT	CCA	GAC	ACT	GTG	192
																40

Ala	Tyr	Ile	Ser	Ser	Gly	Gly	Gly	Gly	Thr	Tyr	Tyr	Pro	Asp	Thr	Val		
50						55					60						
AAG	GGC	CGA	TTC	ACC	ATC	TCT	AGA	GAC	AAT	TCC	AAG	AAC	ACC	CTG	TTC	240	
Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Lys	Asn	Thr	Leu	Phe		
65					70				75					80			
CTG	CAG	ATG	GAC	AGT	CTG	AGG	CCT	GAG	GAC	ACA	GGC	GTG	TAT	TTC	TGT	288	
Leu	Gln	Met	Asp	Ser	Leu	Arg	Pro	Glu	Asp	Thr	Gly	Val	Tyr	Phe	Cys		
				85					90					95			
GCC	CGC	GGG	GGG	GTA	CGA	CGA	GGG	TAC	TTC	GAT	GTC	TGG	GGC	CAA	GGT	336	
Ala	Arg	Gly	Gly	Val	Arg	Arg	Gly	Tyr	Phe	Asp	Val	Trp	Gly	Gln	Gly		
				100				105					110				
ACC	CCG	GTC	ACC	GTC	TCC	TCA										357	
Thr	Pro	Val	Thr	Val	Ser	Ser											
						115											

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 119 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Val	Val	Gln	Pro	Gly	Arg		
1				5					10					15			
Ser	Leu	Arg	Leu	Ser	Cys	Ser	Ser	Ser	Gly	Phe	Ile	Phe	Ser	Ser	Tyr		
			20					25					30				
Asp	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val		
			35				40					45					
Ala	Tyr	Ile	Ser	Ser	Gly	Gly	Gly	Gly	Thr	Tyr	Tyr	Pro	Asp	Thr	Val		
	50					55					60						
Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Lys	Asn	Thr	Leu	Phe		
65					70					75					80		
Leu	Gln	Met	Asp	Ser	Leu	Arg	Pro	Glu	Asp	Thr	Gly	Val	Tyr	Phe	Cys		
				85					90					95			
Ala	Arg	Gly	Gly	Val	Arg	Arg	Gly	Tyr	Phe	Asp	Val	Trp	Gly	Gln	Gly		
			100					105					110				
Thr	Pro	Val	Thr	Val	Ser	Ser											
						115											

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 321 base pairs
 (B) TYPE: nucleic acid

(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..321

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAT ATC CAG ATG ACT CAG TCT CCG AGC TCC CTA TCT GCA TCT GTG GGA	48
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly	
1 5 10 15	
GAC CGT GTC ACC ATC ACG TGT CGA GCA AGT GGG AAT ATT CAC AAT TAT	96
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gly Asn Ile His Asn Tyr	
20 25 30	
TTA ACA TGG TAT CAG CAG ACC CCG GGA AAA GCT CCT AAG CTC CTG ATC	144
Leu Thr Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile	
35 40 45	
TAT AAT GCA AAA ACG CTA GCA GAT GGT GTG CCG TCT AGA TTC AGT GGC	192
Tyr Asn Ala Lys Thr Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly	
50 55 60	
AGT GGA TCA GGA ACA GAT TAT ACT TTC ACG ATC TCA AGC TTG CAG CCT	240
Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro	
65 70 75 80	
GAA GAT ATT GCG ACT TAT TAC TGT CAA CAT TTT TGG AGT ATT CCG TAC	288
Glu Asp Ile Ala Thr Tyr Tyr Cys Gln His Phe Trp Ser Ile Pro Tyr	
85 90 95	
ACG TTC GGC CAA GGG ACC AAG CTG CAA ATA ACT	321
Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr	
100 105	

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gly Asn Ile His Asn Tyr
20 25 30
Leu Thr Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Asn Ala Lys Thr Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
42

50 55 60
 Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80
 Glu Asp Ile Ala Thr Tyr Tyr Cys Gln His Phe Trp Ser Ile Pro Tyr
 85 90 95
 Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr
 100 105

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 81 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 22..81

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GAATTCGAGG ACGCCAGCAA C ATG GTG TTG CAG ACC CAG GTC TTC ATT TCT 51
 Met Val Leu Gln Thr Gln Val Phe Ile Ser
 1 5 10
 CTG TTG CTC TGG ATC TCT GGT GCC TAC GGG 81
 Leu Leu Leu Trp Ile Ser Gly Ala Tyr Gly
 15 20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Val Leu Gln Thr Gln Val Phe Ile Ser Leu Leu Leu Trp Ile Ser
 1 5 10 15
 Gly Ala Tyr Gly
 20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Glu Lys Arg Phe Val Phe
1 5

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CTAACTCA TTCCTGTTGA AGCTCTTGAC AATGGG

36

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTACATATGC AAGGCTTACA ACCACAATC

29

(2) INFORMATION FOR SEQ ID NO:14

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14

CTCCGCGTCG ACGAGCTCCA GATGACTCAG TCTCC

35

(2) INFORMATION FOR SEQ ID NO:15

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs

44

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15

CTCCGCGTCG ACTCTAGACC TAACACTCAT TTC

33

(2) INFORMATION FOR SEQ ID NO:16

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16

CTCCGCGTCG ACCTCGAGTC TGGGGGAGGC

30

(2) INFORMATION FOR SEQ ID NO:17

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 33 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17

CTCCGCGTCG AACTAGTAC AATCCCTGGG CAC

33

(2) INFORMATION FOR SEQ ID NO:18

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18

TCACTGTGCC CGGGGTGGTG TACGACGAGG

30

(2) INFORMATION FOR SEQ ID NO:19

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 25 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19

GGTTGAGGCA GGTCAGACGA TTGGC

25

(2) INFORMATION FOR SEQ ID NO:20

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 48 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20

TATCACTGTG CCCGGGGTGG TNNSNNSNN SGGGNNSTTCG ATGTCTGG

48

(2) INFORMATION FOR SEQ ID NO:21

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21

GTGGTGTAAC CAAGGGGTAC TTCG

24

WHAT IS CLAIMED IS:

1. A fusion protein comprising an amino acid sequence having the antigen specificity of an IL-1 β antibody fused to a selected second protein sequence or fragment thereof.
2. The protein according to claim 1 wherein said antibody is SK48-E26.
3. The protein according to claim 1 wherein said second sequence is heterologous to said sequence having the antigen specificity of an IL-1 β antibody.
4. The protein according to claim 1 wherein said antigen specificity is directed against the amino acid sequence of the human IL-1 β protein spanning amino acid #95 through #101 SEQ ID NO: 11 and analogs or modifications thereof.
5. The protein according to claim 4 wherein said modifications occur within at least one of the CDR regions of SEQ ID NOs: 2 and 4.
6. The protein according to claim 5 wherein said modifications occur within the CDR3 region of SEQ ID NO: 2.
7. The protein according to claim 6 wherein said modification occurs for at least one of the following positions: 120, 121, 122 or 124 of SEQ ID NO: 2.
8. The protein according to claim 1 wherein said amino acid sequence is selected from the group consisting of the heavy chain sequence of SEQ ID NO: 2, the variable region of SEQ ID NO: 2, at least one CDR of SEQ ID NO: 2, and a functional fragment or analog thereof.
9. The protein according to claim 1 wherein said amino acid sequence is selected from the group consisting of a light chain sequence of SEQ ID NO: 4, the variable region of SEQ ID NO: 4, at least one CDR of SEQ ID NO: 4, and a functional fragment or analog thereof.

10. The protein according to claim 1 wherein said amino acid sequence comprises one or more CDR peptides selected from the group consisting of
SYDMS, amino acids #50-54 of SEQ ID NO: 2;
YISSGGGGTYYPDTVKG, amino acids #69-85 of SEQ ID NO: 2;
GGVRRGYFDV, amino acids #118-127 of SEQ ID NO: 2;
RASGNIHNYLT, amino acids # 44-54 of SEQ ID NO: 4 ;
NAKTLAD, amino acids # 70-76 of SEQ ID NO: 4;
QHFWSIPYT, amino acids # 109-117 of SEQ ID NO: 4; and an analog of the above-listed sequences.

11. A fusion protein comprising an amino acid sequence having the antigen specificity of an IL-1 β antibody operatively linked to a selected effector agent.

12. The protein according to claim 11 wherein said effector agent is selected from the group consisting of a non-protein carrier molecule, polystyrene, plastic beads, a macrocycle or a toxin.

13. A fusion molecule comprising a first fusion partner nucleotide sequence encoding an amino acid sequence having the antigen specificity of an IL-1 β antibody operatively linked to a selected second fusion partner nucleotide sequence or an effector agent.

14. The fusion molecule according to claim 13 wherein said first fusion partner nucleotide sequence is selected from the group consisting of the nucleotide sequence encoding a heavy chain sequence selected from the group consisting of SEQ ID NO: 2, the nucleotide sequence encoding the variable region of SEQ ID NO: 2, the nucleotide sequence encoding at least one CDR of SEQ ID NO: 2, the nucleotide sequence encoding the light chain sequence of SEQ ID NO: 4, the nucleotide sequence encoding the variable region of SEQ ID NO: 4, the nucleotide sequence encoding at least one CDR of SEQ ID NO: 4, and a functional fragment or analog or modification of any of the above sequences.

15. The fusion molecule according to claim 14 wherein said modification occurs within at least one of the CDR regions of SEQ ID NO: 2 and SEQ ID NO: 4.

16. The fusion molecule according to claim 15 wherein said modification occurs within the CDR3 region of SEQ ID NO: 2.

17. The fusion molecule according to claim 16 wherein said modification occurs for at least one of the following positions: 120, 121, 122 or 124 of SEQ ID NO: 2.

18. An anti-IL-1 β CDR peptide selected from the sequence of claim 10, and a fragment thereof, or an analog thereof, characterized by the antigen specificity of the SK48-E26 antibody.

19. An isolated IL-1 β antibody heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, the variable region of SEQ ID NO: 2, and a fragment or analog thereof characterized by the anti-IL-1 β antigen specificity of SK48-E26 antibody.

20. An isolated IL-1 β antibody light chain amino acid sequence selected from the group consisting of SEQ ID NO: 4, the variable region of said sequence, and a fragment or analog thereof characterized by the anti-IL-1 β antigen specificity of SK48-E26 antibody.

21. An isolated nucleic acid sequence selected from the group consisting of

- (a) the heavy chain sequence of SEQ ID NO: 1;
- (b) the light chain sequence of SEQ ID NO: 3;
- (c) a variable region of (a) or (b);
- (d) at least one CDR-encoding sequence of (a) or (b); and
- (e) a fragment, modification or analog of (a) or (b),

the sequence encoding a protein or fragment characterized by anti-IL-1 β antigen specificity, and said sequence optionally containing a restriction site to facilitate insertion into a desired antibody framework region or fusion with a selected fusion partner.

22. An engineered antibody comprising an amino acid sequence in which at least parts of the heavy chain variable region of an acceptor antibody have been replaced by analogous parts of the heavy chain variable region of at least one donor antibody having specificity for IL-1 β and a suitable light chain sequence, said acceptor antibody being heterologous to said donor antibody.

23. The antibody according to claim 22 wherein the variable heavy chain region of the donor antibody is fused to the heavy chain constant region of the acceptor antibody.

24. The antibody according to claim 22 wherein the heavy chain CDR fragments of the donor antibody replace the heavy chain CDR fragments of the acceptor antibody.

25. The antibody according to claim 22 wherein the light chain is selected from the group consisting of

- (a) a variable light chain region of the donor antibody fused to the light chain constant region of the acceptor antibody;
- (b) a light chain comprising light chain CDR fragments of the donor antibody replacing the light chain CDR fragments of the acceptor antibody;
- (c) the donor antibody light chain; and
- (d) a heterologous acceptor antibody light chain.

26. The antibody according to claim 22 wherein the variable light chain region of the donor antibody is that of SEQ ID NO: 2 or a functional fragment or modification thereof and the variable heavy chain region of the donor antibody is that of SEQ ID NO: 4, or a functional fragment or modification thereof, wherein the resulting engineered antibody is characterized by the antigen binding specificity of mAb SK48-E26.

27. The engineered antibody according to claim 26, wherein said functional fragment comprises at least one nucleic acid sequence encoding at least one CDR peptide selected from the group consisting of

SYDMS, amino acids #50-54 of SEQ ID NO: 2;

YISSGGGGTYYPDTVKG, amino acids #69-85 of SEQ ID NO: 2;
GGVRRGYFDV, amino acids #118-127 of SEQ ID NO: 2;
RASGNIHNYLT, amino acids # 44-54 of SEQ ID NO: 4 ;
NAKTLAD, amino acids # 70-76 of SEQ ID NO: 4;
QHFWSIPYT, amino acids # 109-117 of SEQ ID NO: 4; and an analog of the
above-listed sequences.

28. The antibody according to claim 26 wherein said modification occurs within at least one of the CDR regions of SEQ ID NO: 2 and SEQ ID NO: 4.

29. The antibody according to claim 28 wherein said modification occurs within at least one CDR3 region of SEQ ID NO: 2.

30. The antibody according to claim 29 wherein said modification occurs for at least one of the following positions: 120, 121, 122 or 124 of SEQ ID NO: 2.

31. The antibody according to claim 30 wherein position 120 is valine and position 124 is tyrosine.

32. The antibody according to claim 31 wherein the amino acid at position 121 of SEQ ID NO: 2 is selected from the group consisting of arginine, histidine and threonine.

33. The antibody to claim 32 wherein position 121 of SEQ ID NO: 2 is threonine.

34. The antibody according to claim 31 wherein the amino acid at position 122 of SEQ ID NO: 2 is arginine or lysine.

35. The antibody according to claim 34 wherein position 122 of SEQ ID NO: 2 is lysine.

36. The antibody according to claim 22 which is capable of binding to the tertiary conformational site of the IL-1 β protein comprising the amino acid sequence

spanning amino acid #95 through #101 SEQ ID NO: 11 humanized recombinant IL-1 β and analogs thereof, a Fab fragment thereof, or an F(ab')₂ fragment thereof, said antibody being a monoclonal antibody or an engineered humanized antibody.

37. A humanized antibody comprising an amino acid sequence in which at least parts of the sequence of the heavy chain of a human acceptor antibody have been replaced by analogous parts of the amino sequence of the heavy chain of at least one IL-1 β donor antibody, and a suitable light chain sequence, said humanized antibody characterized by the antigen specificity of the donor antibody.

38. The antibody according to claim 37 wherein the donor antibody is SK48-E26.

39. The antibody according to claim 37 wherein the heavy chain variable region sequences are SEQ ID NOS: 5 and 6 and the light chain variable region sequences are SEQ ID NOS: 7 and 8.

40. An IL-1 β antibody, SK48-E26, or a functional fragment or analog or modification thereof.

41. The antibody according to claim 40 wherein said modification occurs within at least one of the CDR regions of SEQ ID NO: 2 and SEQ ID NO: 4.

42. The antibody according to claim 41 wherein said modification occurs within the CDR3 region of SEQ ID NO: 2.

43. The fusion molecule according to claim 42 wherein said modification occurs for at least one of the following positions: 120, 121, 122 or 124 of SEQ ID NO: 2.

44. An IL-1 β antibody, a Fab fragment or a F(ab')₂ fragment thereof produced by screening an antibody library comprising hybridoma products and libraries derived from any species immunoglobulin repertoires, with SK48-E26.

45. A pharmaceutical composition comprising an antibody which is capable of binding to amino acids #95-101 (SEQ ID NO: 11) of the IL-1 β protein, and analogs thereof, a Fab fragment thereof, or an F(ab')₂ fragment thereof, said antibody being a monoclonal antibody or an engineered humanized antibody, and a pharmaceutically acceptable carrier or diluent.

46. The pharmaceutical composition according to claim 45 wherein said antibody is SK48-E26.

47. A pharmaceutical composition comprising the antibody of claim 22 and a pharmaceutically acceptable carrier or diluent.

48. A method of preventing or treating an IL-1 β -mediated disorder in a human need thereof which comprises administering to said human an effective dosage of a pharmaceutical composition of claim 47.

49. A recombinant plasmid comprising a nucleic acid sequence of claim 21.

50. A mammalian cell line transfected with the recombinant plasmid of claim 49.

51. A method for producing an anti-IL-1 β fusion proteins, engineered antibodies and Fab fragments comprising culturing a selected host cell transfected with a recombinant plasmid comprising a nucleic acid sequence of claim 13 under the control of selected regulatory sequences capable of directing the expression thereof in said cells.

52. A method of diagnosing IL-1 β -mediated disorders comprising the step of contacting a biological sample from an animal suspected of having an IL-1 β -mediated disorder with a fusion protein according to claim 1.

53. A method of diagnosing IL-1 β -mediated disorders comprising the step of contacting a biological sample from a human suspected of having an IL-1 β -mediated disorder with the antibody of claim 40.

NEUTRALIZING EFFECT OF PURIFIED
SK48E-26.2.19 IgG VS IL-1 ALPHA AND BETA BY BIOASSAY

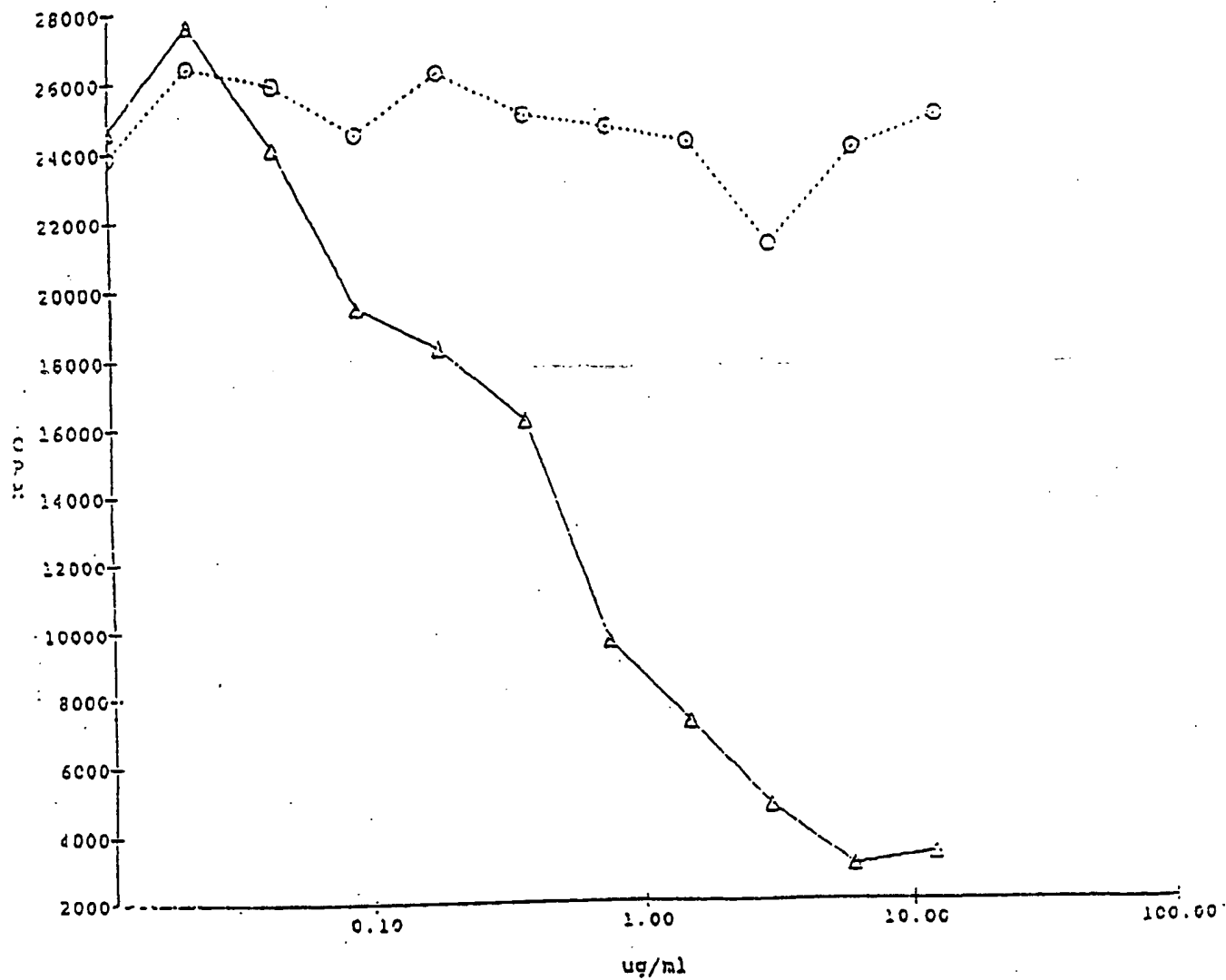


Figure 1

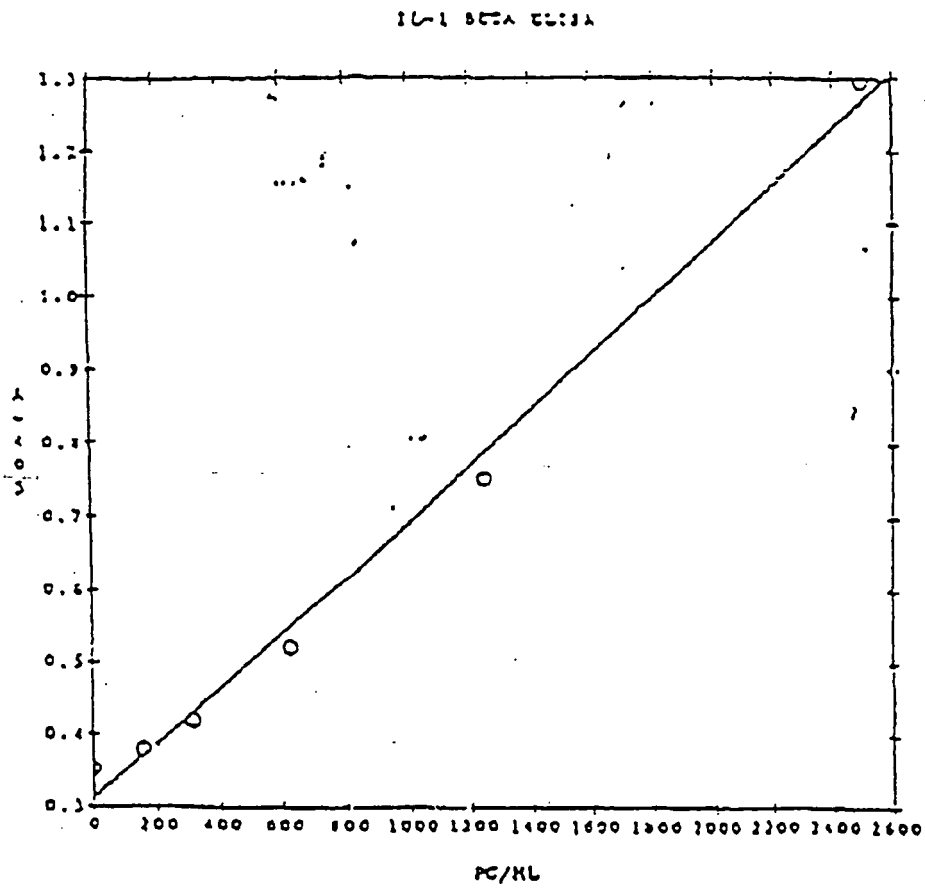


Figure 2

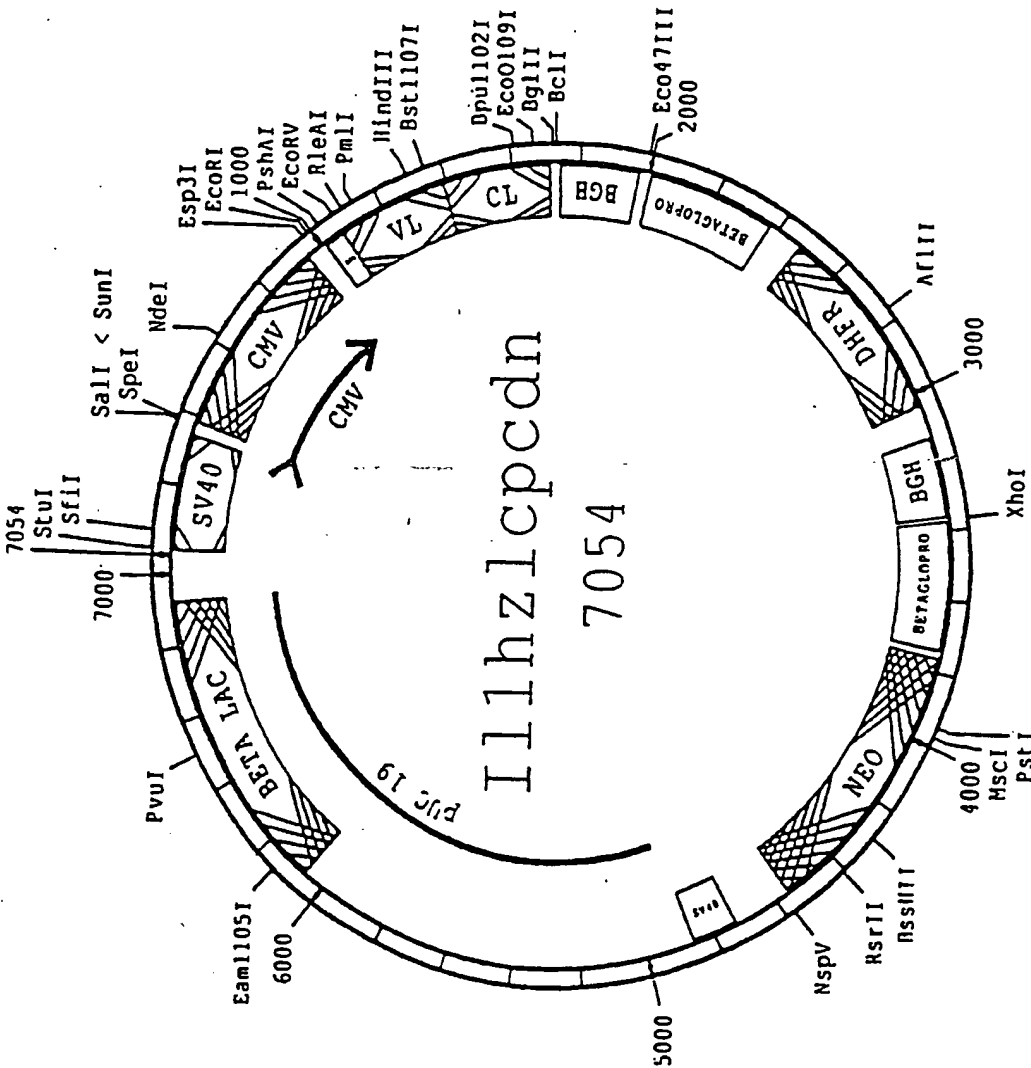


Figure 4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/07659

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/387.3, 388.23, 391.7; 536/23.53; 424/133.1, 145.1; 435/320.1, 240.2, 7.21, 70.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, BIOSIS, FAST DB

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,935,343, (ALLISON ET AL.) 19 JUNE 1990, SEE ENTIRE DOCUMENT.	1-53
Y	WO, A, 91/09967, (ADAIR ET AL.), 11 JULY 1991, SEE PAGES 11, 16.	1-53
Y	EP, A, 0,364,778, (UETSUKI ET AL.) 25 APRIL 1990, SEE COL. 2, 6, 7.	1-53
Y	EP, A, 0,267,611, (OHMOTO ET AL.) 18 MAY 1988, SEE PAGES 2, 9, 10.	1-53
Y	THE JOURNAL OF BIOLOGICAL CHEMISTRY, VOLUME 268, NUMBER 13, ISSUED 05 MAY 1993, SIMON ET AL., "MAPPING OF NEUTRALIZING EPITOPES AND THE RECEPTOR BINDING SITE OF HUMAN INTERLEUKIN 1B", PAGES 9771-9779, SEE PAGE 9771.	1-53



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

19 AUGUST 1994

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SEP 06 1994

Name and mailing address of the ISA/US
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Authorized officer

PAULA HUTZELL

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/US94/07659**C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BIOTECHNIQUES, VOLUME 5, NUMBER 7, ISSUED OCTOBER 1987, GAFFNEY ET AL., "ENZYME LINKED IMMUNOASSAY WITH MONOCLONAL ANTIBODY FOR HUMAN INTERLEUKIN-1-BETA", PAGES 652-659, SEE ENTIRE DOCUMENT.	1-53

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/07659

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (5):

C07K 15/28, C07H 15/00, A61K 39/395, C12N 15/00, C12N 5/10, C12P 21/00, G01N 33/53

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

530/387.3, 388.23, 391.7; 536/23.53; 424/133.1, 145.1; 435/320.1, 240.2, 7.21, 70.1

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- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
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